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THE LIFE CYCLE OF *LEPTORHYNCHOIDES THECATUS* (LINTON), AN ACANTHOCEPHALAN OF FISH[1]

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Leptorhynchoides thecatus is a member of the phylum ACANTHOCEPHALA, the class METACANTHOCEPHALA, the order PALAEACANTHOCEPHALA and the family RHADINORHYNCHIDAE. The members of this family, with the exception of the genus *Leptorhynchoides,* occur as parasites within the enteric canal of marine fish. The genus *Leptorhynchoides* includes forms which parasitize marine fish, and also forms which are found only as parasites of fresh-water fish.

Leptorhynchoides thecatus occurs as a widespread parasite of fresh-water fish in the United States. It is able to utilize a great variety of fish as definitive hosts and is known to develop to maturity in such unrelated families of fish as the CENTRARCHIDAE, CATOSTOMIDAE, PERCIDAE, ESOCIDAE, CYPRINIDAE, and ANGUILLIDAE. Despite this wide host range, if numbers and degree of infection are considered,the large mouth bass, *Huro salmoides,* the small mouth bass, *Micropterous dolomieu,* and the rock bass, *Ambloplites rupestris,* of the family CENTRARCHIDAE, constitute the more normal hosts of this parasite. For a specific diagnosis of the adults of this form the reader is referred to Van Cleave (1934). The reader who desires to extensively review the older literature on the life cycle and development of other species of ACANTHOCEPHALA is referred to the excellent monographs by Hamann (1891), Kaiser (1893), and Meyer (1933).

Up to the present very little was known concerning the life cycle of *Leptorhynchoides thecatus.* Van Cleave (1920) reported finding the juvenile stage of this parasite as a natural infection in the amphipod, *Hyalella azteca.* The same author (1934) stated he was able to experimentally infect *Hyalella azteca* with the eggs of *Leptorhynchoides thecatus,* but gave no further information on development within the amphipod host or infection of the fish.

The present work presents the complete life cycle of *Leptorhynchoides thecatus* as it occurs in the laboratory under controlled experimental conditions, utilizing the amphipod, *Hyalella azteca,* as the intermediate host and the rock bass, *Ambloplites rupestris,* as the definitive host.

MATERIALS AND METHODS

At the onset of the present work the author reared the amphipod intermediate host, *Hyalella azteca,* in the laboratory using the following method: female amphipods

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carrying embryos were collected from nature, brought into the laboratory, placed in battery-jar aquaria, and allowed to drop the young *Hyalella*. The females were then separated from the young and discarded. The young *Hyalella* were placed in fresh cultures made up of well water and willow rootlets in battery-jar aquaria. Although successful, this procedure proved to be laborious, time-consuming, and uncertain. As the work progressed and the author became familiar with the developmental stages of *Leptorhynchoides thecatus* it was found that natural infections could readily be distinguished from experimentally induced infections. Examination of the amphipod, *Hyalella azteca,* collected from nature revealed the intensity of natural infection to be very low in the majority of situations. For example, examination over a period of three years of approximately one thousand amphipods yielded only four natural infections. For these reasons the method of rearing *Hyalella* in the laboratory was abandoned and *Hyalella* collected from nature were used in all later work.

The following method was used to keep *Hyalella azteca* in the laboratory. Specimens were brought into the laboratory, freed of debris, and washed in running well water. The *Hyalella* thus treated were then placed in battery jars containing well water, willow rootlets, and *Anacharis* sp. (*Elodea*). Well water was added periodically to replace loss by evaporation and a small amount of brewer's yeast was added to the cultures each week. The cultures were kept at a temperature of approximately 25° C. and out of direct light. Decomposition was kept at a minimum by constantly passing a stream of air from the compressed air line through the water. This method of keeping *Hyalella* in the laboratory proved very sucessful. The *Hyalella* reproduced well and deaths remained low.

The mature acanthocephalan eggs used to infect the amphipod intermediate host were removed from females of *Leptorhynchoides thecatus* recovered from pyloric caeca of rock bass. The mature female ACANTHOCEPHALA were removed from the fish, placed in well water and stored in the refrigerator at approximately 4° C. Eggs stored in this manner remained viable for nine months.

Infection of the intermediate host, *Hyalella azteca,* was conducted in the following manner: the mature acanthocephalan females were taken from storage, placed in fresh well water, and the body teased apart with dissecting needles. By this method the eggs were released from the body cavity of the female into the water. Eggs collected from three female ACANTHOCEPHALA were placed in a finger bowl of well water. Approximately one hundred and fifty amphipods were then placed in the finger bowl containing the eggs. The *Hyalella* readily ingest the eggs from the water. In order to study successfully the daily stages of development of the acanthocephalan within the amphipod intermediate host, it is desirable to produce light infections. Heavy infections produce larvae in various stages of growth, making it difficult to follow the true course of development. By limiting the time the *Hyalella* are allowed to feed on the eggs, the degree of infection could be controlled. By allowing a feeding period of one hour, infections of from one to four ACANTHOCEPHALA per *Hyalella* were obtained. This method brought about the infection of only 75% of the *Hyalella* exposed, but had the distinct advantage of producing larvae all approximately in the same stage of development. After the proper length of exposure to the acanthocephalan eggs the *Hyalella* were removed from the egg mixture, placed on a fine mesh screen and thoroughly washed by running well-water

to free them of any acanthocephalan eggs which might have clung to the integument and appendages of the amphipod. This assured that the embryos developing within the *Hyalella* were ingested during the hour allowed for infection. After washing, the *Hyalella* were returned to the battery-jar cultures described previously.

The rock bass, *Ambloplites rupestris,* was chosen as the experimental fish host because: 1) it is one of the important natural hosts, 2) it is readily available, and 3) it is more adaptable to laboratory conditions than either the large mouth bass, *Huro salmoides,* or the small mouth bass, *Micropterus d. dolomieu.* Its food habits are more varied than the latter fish. In the laboratory the rock bass lived very well on a wholly earthworm diet.

The rock bass used as the experimental definitive hosts were collected by means of a fyke net set on a rocky bar on the south shore of Lake Mendota, Madison, Wisconsin. After removal from the net, the fish were taken to the laboratory and placed in aquarium tanks provided with running well water, a stream of air for aeration, and fed earthworms at weekly intervals.

The fish were kept in the laboratory two months before experimental infection. During the course of the two months weekly fecal examinations for acanthocephalan eggs were made. Only those fish not showing the presence of acanthocephalan eggs during the two month period were kept for use as the experimental definitive hosts.

Experimental infections of the rock bass with *Leptorhynchoides thecatus* were conducted in the following manner: The infective juveniles of *Leptorhynchoides thecatus* were dissected out of the amphipod body and placed in well water. The fish to be fed were wrapped in a water-saturated cloth to prevent injury during handling. The infective juveniles were taken up with as little water as possible into a long, medium-bore glass pipette. The pipette was gently guided down the throat into the stomach of the fish. Pressure was applied slowly to the bulb of the pipette and the immature ACANTHOCEPHALA were thus deposited into the fish stomach. This method of feeding caused no injury to the fish and resulted in a minimum amount of regurgitation of fed juveniles. Ten infective juveniles were fed each fish. Immediately after feeding, each fish was placed in a large battery jar aquarium containing well water and allowed to remain isolated in this manner for one hour. This was done to further check for regurgitation of fed juveniles. At the end of the hour the water was carefully examined and if juveniles were found, an equal number were re-fed. The fish were then returned to the tank aquarium.

Methods Used to Study Developmental Stages

A. Stages in the amphipod intermediate host, Hyalella azteca. Insofar as was possible, the developmental stages within *Hyalella azteca* were studied at daily intervals. A number of amphipods from the infected culture were examined each day at approximately the same time. Recovery of the developmental stages for study necessitated the dissection of the amphipod. The amphipod to be examined was placed in a small drop of water on a microscope slide. The slide was then placed on the stage of a binocular dissecting microscope. By using dissecting needles, the last three anal segments of the amphipod were severed and removed, thereby breaking the attachment of the posterior end of the intestine to the body. The amphipod body was then held firmly with one needle. A second needle was forced into the

head and, by applying a gentle pull, the head, carrying with it the digestive glands and digestive tract, was freed from the body. The head, digestive glands, and intestinal canal were then covered with a cover glass, thus allowing them to remain in the mixture of water and amphipod hemocoele fluid. This proved to be a very convenient method for study of early developmental stages which are attached to the amphipod intestine. The intestinal canal remained functional for some time after separation from the body, thus providing a more normal environment for the developing parasite while under observation than if they had been removed to pure tap water. It was especially favorable for the study of the embryo hatching from the egg and free in the intestinal lumen. The intestine of *Hyalella* is very transparent and if freed of food material offers little obstruction during microscopic study of its contents.

In the study of later developmental stages, where the acanthella is free within the hemocoele of the amphipod, the same method of dissection was used. In this case, however, when the amphipod body was opened, the acanthellae escaped into the surrounding hemocoele fluid and water. Here again, the parasite was retained in this medium and studied as a wet mount under a cover glass sealed with petroleum jelly to prevent evaporation.

Whenever possible the developmental stages were studied in the living state and drawn with the aid of a camera lucida. This could readily be done with the acanthor and acanthella in early stages of development. Acanthellae, during late development, did not withstand the manipulation required in using this method; for this reason they were studied only briefly in the living condition. In all cases the specimen studied while living was fixed for later study.

Penetration of the acanthor through the epithelium of the amphipod intestine could not be clearly observed in living material, but was reconstructed from serial sections.

All developmental stages, both from *Hyalella* and, later, the fish, were fixed in hot F.A.A. (according to Guyer's formula, Guyer's *Animal Micrology,* 1936), stained in Semichon's (1924) acetic carmine, and mounted in balsam.

B. Stages in the fish definitive host, Ambloplites rupestris. Because of the difficulty of obtaining uninfected fish and the limitation in the number of fish that could be conveniently handled in the laboratory, the development within the fish host was studied at intervals of one week, two weeks, three weeks, four weeks, six weeks, and eight weeks. At each of these intervals one of the experimentally infected fish was killed and the intestine examined for the presence of ACANTHOCEPHALA. As all of the fish were originally given ten larvae, the number found would serve as a check for the presence of natural infections. In none of the experimentally infected fish was the number recovered above or equal to the number of infective larvae fed. The largest number, nine, was recovered from the fish killed four weeks after infection. The lowest number recovered was five individuals. In all cases both males and females were present. Except for measurements, no attempt was made to study the material recovered from the fish in living condition. The specimens were preserved, stained, and mounted by the method described.

Beginning on the sixth week after infection, weekly examinations of feces of the remaining fish were conducted to find the first date of passage of eggs. When this occurred, the experiment was terminated.

LIFE CYCLE OF *Leptorhynchoides thecatus*

A. Development within H. azteca. The eggs of ACANTHOCEPHALA passed from the mature females contain a fully developed embryo. The egg of *Leptorhynchoides thecatus,* when recovered from fish feces, is a blunt end, spindle-shaped object made up of two distinct shell membranes surrounding a thin, delicate embryonic membrane containing an embryo (Plate I fig 1). The embryo is inactive and hatching does not occur until the egg is ingested by the proper intermediate host.

The amphipod, *Hyalella azteca,* ingests the egg of *Leptorhynchoides thecatus.* The egg passes into the fore-intestine of the amphipod and is subjected to the action of digestive enzymes. The embryo within the egg becomes active and faces its anterior end against the inner embryonic membrane. The action of the digestive enzymes weakens the outer shell membranes. The movements of the embryo rupture the membranes at the anterior end of the egg and the embryo escapes into the lumen of the amphipod intestine. Hatching requires approximately three-quarters of an hour and appears to be brought about by the combined action of the digestive enzymes of the amphipod and movements of the embryo.

The recently liberated acanthor is elongate with rounded anterior and posterior extremities. The anterior end is provided with fourteen rows of stout, backward-pointing spines, five in each row (Plate I fig 2). Subterminally, a small area of the anterior end can be drawn in to form a cup-like structure into which host tissue is drawn (Plate I fig 5). A rostellum with blade-like hooks could not be demonstrated in this form. The posterior extremity of the embryo is provided with a variable number of small, inconspicuous spines. The acanthor, free in the intestine of *Hyalella,* is actively motile. It moves along the surface of the host epithelium in the following fashion: The embryo extends the body, grasps the epithelium in the anterior pouch and draws the rear portion of the body forward. When free in liquid media, the movements result only in aimless flexion and extension of the body, not followed by forward movement. The morphology of the recently-hatched acanthor is very simple. It is made up of a centrally-located, compact embryonic nuclear mass, surrounded by a protoplasmic syncytium which contains fourteen giant nuclei, only faintly visible in the living embryo. The whole is surrounded by a delicate pellicle which bears the larval spines. The central embryonic nuclear mass is the entoblast and is the anlage for the majority of internal adult structures. The peripheral syncytial layer containing the giant nuclei is the ectoblast destined to give rise to all adult cortical structures (Plate I fig. 2).

After a short period of wandering within the gut of the amphipod, the recently-hatched acanthor attaches itself and begins to penetrate the epithelium (Plate I fig 3). The acanthor makes its way through the epithelial cells and locates between the basal membrane of the epithelium and the serosa of the host intestine (Plate I fig 4). Having completed penetration, the acanthor ceases all motility and enters the acanthella stage. There now begins a period of peculiar growth, characterized by a bulging outward of the central portion of the acanthor body situated against the host serosa (Plate I fig 5, 6, and 7). As growth continues the bulge becomes a round, bladder-like structure projecting into the hemocoele of the host and is attached to the host intestine by the original acanthor body (Plate I fig 9). The host serosa originally covering the acanthor is greatly distended and covers

the bladder-like growth. During this period of growth internal changes have taken place. The central embryonic nuclear mass is now located centrally in the bladder-like portion of the developing acanthella. Proliferation of its elements has taken place. Its appearance has changed from a compact mass of nuclear material to a more loose aggregation of distinct nuclei (Plate I fig 9 and 10). The cortical giant nuclei are now arranged in the syncytial protoplasm at the periphery of the bladder-like form. This peripheral protoplasm has become coarsely granular in nature and contains vacuole-like structures. Nine days after infection the body of the acanthella has attained a diameter of 87 microns. The serosa covering the bulge has ruptured and has constricted down around the area where the bladder-like portion of the body joins with the body of the original acanthor. Also at this period the peripheral syncytial protoplasm has become distinctly separated from the central embryonic nuclear mass by what appears to be a delicate plasma membrane (Plate I fig 9 and 10). The developing bladder-like body of the acanthella is now projecting freely into the hemocoele, but retains its attachment to the host intestine by means of the original acanthor body.

The period between the ninth and fourteenth day after infection is characterized by great growth in size of the acanthella and organization of the central nuclear mass (Plate II fig 14, 15, and 17). Beginning on the twelfth day after infection, the rounded acanthella draws outward from its attachment to the body of the original embryo and assumes a tear-drop shape (Plate II fig 14). By the thirteenth day the larval body within the hemocoele of the host is connected to its attachment by a definite stalk (Plate II fig 14). The formerly round body has become slightly ellipsoidal (Plate II fig 14). Concurrent with these external changes the central embryonic mass similarily elongates. The body of the acanthella after fourteen days of development has become definitely ellipsoidal. The central nuclear mass has elongated. The nuclear elements at the ends of the mass have enlarged and appear as zones separate from the central smaller nucleated portion. The nuclei at one end are larger than those of the opposite end. From later developmental stages it can be shown that this end, made up of the larger nuclear elements, is destined to become the anterior extremity of the future adult acanthocephalan. The enlarged nuclear elements at this extremity of the central mass constitute the anlage of the adult proboscis. The slightly smaller nuclear elements at the opposite end of the central mass represent the anlage of the internal parts of the posterior portion of the future adult reproductive systems (Plate II fig 17).

It can thus be seen that on the fourteenth day of larval development the future adult body axis has become established.

During this period external changes have also taken place. The stalk attaching the parasite to the host intestine has progressively elongated and become thinner. The protoplasm contained in the remnants of the original acanthor body has been drawn up into the developing acanthella. Late on the fourteenth day the stalk breaks and the acanthella floats freely within the hemocoele of the intermediate host. The 15–16-day acanthella has a broadly elliptical shape and measures 280 microns in length and 208 microns in breadth. The separation of the cortical layer and medullary layer of the body is very distinct. The cortical layer is very broad and is made up of granular syncytial protoplasm. The giant cortical nuclei are large, rounded and vesicular, and have not as yet become established in a set position within

the cortical syncytium. The central nuclear mass has elongated and extends nearly the full length of the body. Differentiation of its elements into the anlage of future adult structures is taking place (Plate II fig 18). The anterior half of this central mass has differentiated into a flask-like body destined to become the proboscis sheath of the adult. The posterior enlarged portion of the developing proboscis sheath is occupied by a mass of myoblast nuclei, which are to give rise to the muscular walls of the sheath and retractor muscles of the proboscis. Centrally located within the myoblast nuclear mass is the anlage of the future brain. The elongate neck-like portion of the proboscis sheath is occupied by the developing proboscis. Attached to the extreme posterior end of the developing sheath are the anlage of the dorsal and ventral retractor muscles of the proboscis sheath. The remaining half of the central nuclear mass of the late fifteen day acanthella contains the anlagen of the body wall musculature, of the gonads, and of the other reproductive organs (Plate II fig 19).

From the fifteenth to the twenty-fifth day of development in the amphipod host the basic architecture of the adult is completed. The development following this period is an elaboration of structure already present in rudimentary form within the acanthella. The acanthella at seventeen days of age is elongate and cylindrical. It measures 523 microns in length and 196 microns in breadth. The cortical layer has become reduced to a narrow band surrounding the central structures. The giant cortical nuclei have elongated and are definitely placed within the cortical layer. Four of these giant nuclei have formed a ring at the anterior end of the larva, girdling the site around the developing proboscis. They mark the posterior end of the adult proboscis (or neck) where the proboscis and proboscis-sheath become continuous. Eight of the giant nuclei are located in the cortical layer of the body, arranged four on each side and enter into the formation of the future cortex and cuticula. The remaining two giant nuclei are located one at the extreme anterior end of the acanthella forming a cap over the anterior end of the developing proboscis; the other, at the extreme posterior tip of the acanthella below the anlage of the posterior portion of the reproductive system. It enters into the formation of the cuticular portion of the bursa in the male, and the vagina in the female. The medullary layer of the seventeen-day acanthella has become greatly elongated and has pulled away from the cortical portion. It now projects as a core through the center of the larva, separated from the cortex by a cavity. This cavity or space does not represent the ligament sacs of the adult. The central core of tissue has begun to split into two layers: an outer layer which comes to lie next to the cortical wall and will give rise to the circular and longitudinal muscle fibers of the adult body wall; the central layer which remains in position gives rise to the genital ligament, gonads, glands, and other internal parts of the reproductive system (Plate III fig 25).

After eighteen days of development, the splitting of the medullary core is completed. The ligament sacs of the future adult are now present, being the space surrounded by the myoblast nuclei which give rise to the body wall musculature (Plate III fig 25). The genital ligament is at this stage distinct, projecting from the posterior end of the proboscis sheath through the body cavity to the posterior end of the body. The genital ligament of the eighteen-day acanthella is thickly nucleated throughout its length. The anlagen of structures destined to develop within the ligament appear blocked out as dense nuclear agglomerations.

These are the anlagen of the testes and cement glands in the male. The muscular bulb of the bursa and the penis are at this stage well outlined. In the female the anlage of the ovary appears very late in development and is not discernible on the eighteenth day of development, but the uterine bell, or selector apparatus, and the uterus are clearly outlined.

1. Development of the reproductive system. For clarity and ease of description, development of the parasite beyond eighteen days will be discussed from the standpoint of organ systems. Both the male and female reproductive systems develop along comparatively similar lines. Thus, in discussing the reproductive systems, the development of the male system will be considered in detail first and later the female system will be compared to that of the male.

In the acanthella during the late fifteen-day stage of development, the central cellular mass, although it has not yet split into two portions which are to give rise to the body musculature and reproductive organs, has become sufficiently differenciated to show where splitting is to occur. It is made up of a peripheral, loosely arranged layer of cells and a central core of more compact cells. This central compact core is further differentiated along its length into distinct groups of cells. These can be recognized as the anlagen of the future reproductive organs. During this stage of development the sex of the acanthella can not be distinguished. As development continues through the sixteenth and seventeenth days splitting of the central medullary mass has begun. The anlage of the body musculature has moved laterally, thus separating from the central anlage of the reproductive system. This anlage of the reproductive system is a rather broad band of cells extending from the posterior tip of the proboscis sheath to the extreme posterior end of the larval body. Differentiation into zones has occurred along its length. It is made up of a short anterior zone of a rather narrow band of nuclei attached to the proboscis sheath which gives rise to the genital suspensory ligament. Just posterior to this a second zone of larger cells can be seen. By proliferation they have formed a mass which bulges outward on each side. This group of cells constitutes the anlage of the testes. Just posterior to the anlage of the testes there is a short narrow band of small compact cells followed by another zone of large cells which are the anlage of the cement glands. Just posterior to the anlage of the cement glands, two very large cells have separated and are arranged side by side. As development proceeds into the late seventeenth day these two cells have fused at their adjacent sides, forming a rounded structure with a central pointed indentation. This structure is the anlage of the muscular bulb of the male bursa and the future penis. The giant cortical nucleus at the extreme posterior tip of the body has, by the seventeenth day of development, broken up into numerous small nuclei arranged in a small mass in contact at its anterior end with the posterior edge of the anlage of the muscular portion of the bursa. This group of nuclei constitutes the anlage of the protrusible cuticula-lined bell of the adult bursa (Plate III fig 25).

During the eighteenth, nineteenth, and twentieth days of development growth in length of the body proceeds rapidly. By the twentieth day the acanthella measures approximately 850 microns in length and 143 microns in breadth. At the end of the eighteenth day of development the lateral anlagen of the body musculature have fused to the cortical wall of the acanthella. The central strand, representing the genital ligament and developing reproductive organs, is now well isolated in the central por-

tion of a space which will be the future adult body cavity. As elongation of the body occurs this central core lengthens correspondingly. This lengthening separates its component parts so that they become more distinct. During the eighteenth day of development the compact anlage of the male testes has begun to separate into two distinct rounded masses which are to become the two testes of the future adult. The cells making up the anlage of the cement glands have increased in size and are arranged in a rosette-like mass. The anlage of the muscular bulb of the bursa has increased in size. It appears as a rounded, transparent body. Internally it has developed two small cavities separated from each other by a broad rod-like structure. This rod-like structure is the anlage of the penis. The anlage of the cuticular portion of the bursa has lengthened and has fused to the posterior end of the muscular bulb. At the end of the nineteenth day of development the portion of the central mass anterior to the testes (the genital ligament) has become narrowed and elongated and shows a decrease in the number of nuclear elements within it. The anlage of the testes has completely separated into two parts which can now be recognized as the anterior and posterior testes of the adult. Just posterior to the testes two strands of cells have become arranged in linear fashion projecting downward to the anlage of the cement glands. These are the developing vasa efferentia. The anlage of the cement glands is now made up of a group of closely packed pear-shaped cells. Just posterior to the developing cement glands is located a compact group of undifferentiated nuclei which represent the anlage of the ducts of the cement glands and Saefligen's pouch. The muscular bulb of the bursa has increased in size, the inner cavity is more pronounced and the penis is very distinct. The cuticula-lined portion of the bursa has lengthened and projects into the ligament sacs. It has begun to lose its compact nuclear appearance. The nuclei are beginning to coalesce, forming fewer but larger nuclear bodies. By the end of the twentieth day of development the anterior portion of the central mass has become an elongated, transparent ligament-like structure; the nuclei formerly present in it have disappeared and only two are discernible within its length. It is now similar to the genital ligament of the adult. The testes have become compact, slightly elliptical structures, located one anterior to to the other within the genital ligament. The cement glands can now be seen as eight distinct pear-shaped cells. Both the muscular and cuticular portions of the bursa have increased in size. The nuclei of the cuticula-lined portion of the bursa have come together to form three large nuclear masses within the cortical layer at the extreme posterior end surrounding the cuticular portion of the bursa in the region of the male genital opening. The anlage of the muscles of the bursa are now evident. All reproductive organs of the male are now present; development which occurs after the twentieth day consists of growth in size of the structures already present and completion of the musculature of the bursa (Plate I fig 12 and 13). The development of the male reproductive system of the young acanthocephalan is complete after twenty-seven days within the amphipod (Plate IV fig 30 and 31).

The development of the female reproductive system rather closely parallels that of the male with one exception. The genital ligament remains broad and filled with nuclear elements and the anlage of the ovary does not appear as a distinct, condensed body during development within the amphipod. On the sixteenth and eighteenth days of development the female acanthella is very similar to the male in that the medullary mass has begun to split into two portions. The central portion or anlage

of the reproductive system differs from the male in that it has a regular configuration and is not differentiated into distinct zones. Beginning on the eighteenth day of development there can be distinguished in the posterior portion of the genital anlage a group of three large cells located in the same relative position as the anlage of the muscular bulb of the male bursa. Two of these cells lie side by side with the third located above when viewed on the horizontal plane. In cross section they appear as a ring of three disk-like cells standing on edge with a space between them. Their anterior edge is attached to the genital ligament. Just posterior to these three cells are two smaller cells. These are followed posteriorly by a chord of compactly arranged cells marking the posterior limits of the anlage of the female reproductive system. The extreme posterior cortical nucleus has, at this period of development, broken up into a group of small nuclei which extend as a column upward to meet the posterior column of cells of the central portion of the reproductive system. During later stages of development the more anterior group of three cells coalesce to form a structure like the body of a funnel. The two cells directly posterior to this form a stem-like tube which fuses to the body of the funnel. This part becomes the uterine bell or selector apparatus of the adult. The chord of cells located posterior to the developing uterine bell forms an elongated muscular tube, the uterus of the adult. Posterior to this the nuclei originating from the posterior cortical nuclei form a short tube which connects with the uterus and opens to the outside as the vagina (Plate IV fig 26–29).

After twenty-seven days of development the female reproductive system, except for the ovary, is complete. The genital ligament, as previously stated, remains in the larval female as a long, tubular structure filled with nuclear material. This nuclear material represents in part the anlage of the ovary, and does not resolve itself into a compact mass until development in the fish is begun. On the twenty-seventh day of development the posterior giant cortical nucleus is, as in the male, re-formed and is located in the cortical layer which surrounds the vaginal pore of the female.

2. Development of the proboscis. The proboscis of *Leptorhynchoides thecatus* begins its development invaginated within the proboscis sheath. Its rudiment is first discernible during the late stage of the fifteen-day larva. At this time it is present as anterior and posterior groups of anlage cells between which there extends a series of protoplasmic strands. In the larvae fifteen days of age, the posterior group of anlage cells have not as yet detached from the muscle anlage filling the bulk of the proboscis sheath (Plate II fig 19). During the sixteenth and seventeenth days of development this separation becomes complete (Plate III fig 25). The developing proboscis can now be seen through its entire length. The anterior group of anlage cells is located at the anterior limits of the ring of the four cortical giant neck nuclei. The posterior group of anlage cells is located within the proboscis sheath. The proboscis thus invaginated occupies approximately two-thirds of the length of the sheath (Plate III fig 25).

The localization of the ring of giant cortical neck nuclei at the anterior end of the developing larva has divided the larval body into two distinct regions: an anterior region which is given over entirely to the development of the proboscis, and a posterior region projecting behind the ring of giant cortical neck nuclei which forms the body of the adult (Plate I fig 12 and 13, and Plate IV fig 27–29).

Beginning with the eighteenth day of development the region anterior to the ring of giant cortical neck nuclei begins to grow in length. As this anterior region elongates the invaginated, developing proboscis begins to evert. This eversion keeps pace with the growth in length of the anterior region of the larval body. Eversion causes the posterior group of proboscis anlage cells to pass forward in the proboscis sheath (Plate IV fig 26–29). By the twentieth day of development the proboscis is one-third everted (Plate III fig 20, and Plate IV fig 27). The posterior anlage, now made up of distinct cells, is located within the sheath just below the posterior level of the giant cortical neck nuclei (Plate III fig 20). The anterior anlage has also broken up into distinct cells. Its position remains unchanged and has become permanently located to form a ring surrounding the evaginating portion of the proboscis at the extreme anterior level of the giant cortical neck nuclei (Plate III fig 20–23, and Plate IV fig 27–29). These original anlage cells form the posterior limits of the extended proboscis. Also in the twenty-day acanthella, the protoplasmic processes which, during the fifteen, sixteen, and seventeen-day acanthella bridged the gap between the anterior and posterior group of proboscis anlage cells, have developed into distinct ridges or laminae. There are thirteen of these laminae present, arranged side by side to form a potentially hollow tubular rod. These are to form the plates from which arise the future proboscis hooks. The anterior giant cortical nucleus has at this period of development separated into several distinct nuclear bodies (Plate III fig 20). During the period between the twentieth and twenty-second day of development, the anterior end of the acanthella continues to grow in length; the proboscis has further evaginated and is now over one-half everted. The posterior group of anlage cells is now located well above the anterior edge of the ring formed by the giant neck nuclei. The laminae which form the base for the future proboscis hooks are distinctly outlined and have developed along their length a central broad ridge. Along this central ridge a series of swellings has developed. These are the anlage of the proboscis hooks (Plate III fig 20–21). The anterior giant cortical nucleus is at this stage represented by four distinct large nuclei, located directly above the everting proboscis (Plate III fig 21). After twenty-three days development the proboscis has completed eversion. The former posterior group of anlage cells is now located at the extreme anterior end of the proboscis. The swellings on the ridges of the hook laminae have become very pronounced. It can now be seen that each is the precursor of a proboscis hook. On the twenty-third day the anterior giant cortical nucleus has begun to reappear as a single body (Plate III fig 23). By the twenty-fifth day of development the swellings on the ridges of the hook laminae have grown outward as elongated, finger-like processes extending into the cortical layer of the anterior end of the acanthella (Plate III fig 22). Growth of these processes outward continues through the twenty-sixth, twenty-seventh, and twenty-eighth days of development. On the twenty-seventh day the points of the hooks project through the larval cortex. They have become slightly recurved in shape. During the twenty-eighth day the hooks continue to grow outward and become increasingly recurved. At the end of the twenty-ninth day of development the hooks are recurved as in the adult and extend completely through the cuticula. The proboscis is fully developed and is retracted into the proboscis sheath after thirty days of development (Plate III fig 24).

The musculature of the proboscis develops simultaneously with the proboscis. During the sixteenth and seventeenth days, as the posterior anlage of the proboscis separates from the mass of myoblast nuclei present in the proboscis sheath, it draws with it strands of protoplasm which remain attached at their posterior ends to the myoblast nuclei within the sheath. These strands of muscle protoplasm progressively lengthen as the proboscis evaginates. As the strands lengthen, myoblast nuclei migrate into them. By the twentieth day of development these protoplasmic strands have become definite muscle bands. These form the inverter muscles attached to the inner apex of the proboscis and extending downward through the center of the proboscis sheath to its base, where they connect with the fibers of the dorsal and ventral retractor muscles of the sheath (Plate III fig 20–23).

3. General body growth and development of the cortical layer. It has been pointed out that during the sixteenth and seventeenth days of development within the amphipod intermediate host the body of the developing acanthocephalan had become divided into distinct anterior and posterior areas by the localization of the giant cortical neck nuclei. Eight of the original fourteen giant cortical neck nuclei have become arranged in a longitudinal row in the lateral cortical walls. Beginning with the seventeenth day of development these eight laterally placed cortical nuclei elongate. This elongation continues to the twenty-second day of development (Plate III fig 25, and Plate IV fig 26–28). Beginning on the twenty-second day these giant cortical nuclei become irregular in configuration, throwing out short, blunt processes from all sides. Up to the twenty-second day of larval development the cortex has remained a rather uniformly granular syncytial protoplasmic layer. During the twenty-second day of development it starts differentiating into two layers with rather indistinct boundaries. The inner layer bordering the body musculature becomes more compact than an outer, still granular layer. As development progresses into the twenty-third day the giant cortical nuclei have become dendritic with long processes ramifying out from a central broad body (Plate IV fig 29). Proceeding through the twenty-fourth day the processes have become thinner and elongated, extending well over the dorsal and ventral surfaces of the body. The cortex has differentiated into two layers of approximately equal thickness. The inner layer has become fibrillar in appearance. The outer layer still appears granular. During the twenty-fifth and twenty-sixth days of development the cortical nuclei continue to elongate both longitudinally and laterally, and by the twenty-sixth day the bodies of the nuclei have approximated each other longitudinally and now form a narrow lateral band of what appears to be continuous nuclear material (Plate IV fig 31). The lateral ramifications have grown over, girdling the dorsal and ventral surfaces of the acanthella. Nuclear processes of one side do not come in contact with those of the opposite side because of an alternation in arrangement. During this period the inner layer of the cortex has broadened and is made up of radially disposed fibers. The outer layer has thinned and remains granular. On the twenty-seventh day of development the nuclear network begins to break up into individual nuclear fragments (Plate IV fig 30). Through the twenty-eighth and twenty-ninth days of development, fragmentation of the nuclear network has become complete. The fragmented nuclear material moves out into the fibrillar layer of the cortex. The area occupied by the original nuclear network remains as communicating canals within this fibrillar layer. This system of canals forms the canal-

icular system of the adult cortex. This consists of two lateral longitudinal canals giving off branches which extend over the dorsal and ventral surface from each side.

During the twenty-eighth, twenty-ninth, and thirtieth days of development the cortical layer has continued to differentiate. At thirty days of age this cortical layer is made up of three distinct parts: an inner broad layer, radially fibrillar containing the canalicular system and nuclear fragments; a central, thin granular layer; and an outer, extremely thin, compact layer—the cuticula. The cortical layer, except for breadth, is now similar to that of the adult (Plate II fig 19).

Thirty to thirty-two days after its entrance as an embryo into the digestive tract of the amphipod the original embryo of *Leptorhynchoides thecatus* has become a juvenile acanthocephalan, dependent for further development on ingestion by the fish definitive host. It has grown to a body length of from 1.9 to 2.4 mm. and lies free in the hemocoele with the proboscis invaginated. The male has all organ systems, including the reproductive system, developed. Its further development within the fish host consists of growth in size and maturation of the products of the reproductive system. The female has present in completed form all the organs of the adult with the exception of the ovary (Plate IV fig 32). Development within the fish host consists of growth in body size, completion and maturation of the reproductive organs, and production of mature embryos.

B. Development in Fish, A. rupestris. The amphipod intermediate host containing the infective juvenile of *L. thecatus* is ingested by the fish. Digestion of the surrounding amphipod tissue releases the juvenile acanthocephalan into the fish stomach. Within the rock bass host the juvenile acanthocephalan enters the pyloric caeca and attaches to the cæcal wall. Development within the fish proceeds as follows: stages recovered from fish one week after experimental infection differ only slightly from the juvenile present after thirty-two days of development within the amphipod host. Neither the male nor female individuals show an increase in body length. The cuticle and subcuticular layer of the body wall have increased in thickness. In the female the genital ligament loses its nucleated appearance and the ovarian anlage is present as a compact group of nuclei located in the central portion of the ligament. In the male some proliferation of the testicular elements appears to be taking place. This first week of life in the fish seems to be primarily a period of physiological adjustment to a new and changed environment (Plate IV fig 33).

At the end of two weeks after infection, the young acanthocephalans have increased in body length. The male now measures 4 mm. and the female, 5 mm. Changes have occurred in the gonads. In the male the testes have increased in size and are now made up of a mass of distinct, closely packed nuclei. In the female the genital ligament has become a thin cord-like structure suspended from the proboscis sheath anteriorly, bulged midway down its length to surround the ovarian anlage, and attached posteriorly to the uterine bell. The ovarian anlage has doubled in size and is splitting into ball-like structures composed of compactly arranged nuclei (Plate IV fig 34–38).

Young ACANTHOCEPHALA recovered three weeks after experimental infections have continued to increase in size. The males measure approximately 5 mm. in length and the females, 7 mm. In males the testes have increased greatly in size and the great majority of specimens had the bursa everted when recovered from the

fish (Plate IV fig 39). In the three weeks old female the ovarian anlage has greatly lengthened and is broken up into elongate elliptical masses. Some of these masses have escaped from the genital ligament and are free in the body cavity of the female (Plate IV fig 35). The cuticula of both the male and female is now the same as that of the adult.

By the fourth week of development within the fish intestine the male has grown to 8 mm. in length. The vasa efferentia are filled with sperm. The cement glands have been actively secreting. Thus, the male system at four weeks gives every indication of being functional. The male acanthocephalan has by this period attained full body length (Plate IV fig 40).

The female, after four weeks development in the fish, has also attained a length of 8 mm. The ovarian anlage has stopped growth within the genital ligament. The number of ovarian balls floating within the ligament sacs has greatly increased. Each floating ovary is now split into a mass of large rounded nuclei (Plate IV fig 36).

At six weeks of development the ovarian anlage of the female has completely disappeared. The ligament sacs are filled with the floating ovarian masses. These floating ovarian masses contain oocytes in varying stages of development. Floating free in the fluid of the ligament sacs are fertilized eggs just recently liberated from the ovarian masses and embryos in varying stages of development: e.g., very young, spherical embryos; other older embryos, bluntly elliptical in shape; and still older embryos sharply elliptical and enclosed in a thin embryonic membrane. The body of the female has grown to 9 mm. in length (Plate IV fig 37).

Eight weeks after experimental infection, acanthocephalan eggs were recovered from the feces of the rock bass. The females of this age removed from the fish had grown to 1.2 cm. in length. The ligament sacs were closely packed with eggs and embryos in all stages of development from the very young, spherical embryo to the mature embryo invested by its full complement of embryonic membranes. The uteri of the females were filled with mature eggs ready to be passed from the body.

By the fourth week of development within the fish the male has attained maturity. It shows no further increase in size during the six and eight week stages. No definite statement can be made as to the exact time in which copulation and fertilization of the female occurs; however, from the development of the male it can be inferred that fertilization probably takes place during the interval between the third and fourth week of development.

Eggs recovered from eight week old females of the experimentally infected fish were fed to the amphipod intermediate host. Heavy infections were obtained.

The Effect of Temperature on the Development of the Acanthocephalan Within the Amphipod Intermediate Host

The development of *L. thecatus* within the amphipod is affected by the temperature at which the amphipod is kept. During the winter of 1939–40 cultures of experimentally infected *Hyalella* were kept in an unheated aquarium room where the temperature ranged from 13 to 15° C. It was found that at this temperature the development of the acanthocephalan within the amphipod was greatly retarded. Development after penetration proceeded very slowly. Acanthellae, two months after penetration and kept at 13 to 15° C., had grown to a stage comparable in size and

development to acanthellae eight days of age in amphipods infected during the previous summer and kept at room temperature (approximately 25° C.).

A series of experiments were run in which the cultures were kept in a water bath at controlled temperatures. The lowest temperature range used was 13° C The infected amphipods were kept at this temperature for a period of three months. During this period the ACANTHOCEPHALA developed only up to the normal eight to ten day level. Another series of cultures were kept at a temperature which varied from 20 to 25° C. At this temperature infective juveniles developed in from thirty to thirty-two days. Cultures kept at approximately 30° C. showed no increase in developmental rate over those kept at 20 to 25° C., but showed a disadvantage in that mortality among the amphipods at 30° C. was much higher than for those kept at 20 to 25° C.

Cellular Response of the Amphipod Intermediate Host to the Developing Acanthocephalan

The developing acanthocephalan within the amphipod elicits an active cellular reaction from the host tissues. The normal epithelium of the amphipod intestine is one cell in thickness. During penetration of the acanthor through the epithelium, proliferation of the epithelial cells takes place around the acanthor causing a massing of cells at the site of penetration (Plate I fig 3). After the acanthor has completed penetration and begins its bulging growth into the hemocoele, cells from the hemocoele fluid attach themselves to the serosa over the site of the growing parasite. When growth into the hemocoele ruptures the thin amphipod serosa, a cellular reaction takes place which may be likened to the foreign body reaction in higher organisms. Large amoeboid cells from the hemocoele fluid attach themselves to the surface of the parasite body and send out protoplasmic processes which spread over its surface and attach to the amphipod serosa. The protoplasmic processes of the individual cells coalesce to produce a protoplasmic syncytium, in many instances completely investing the parasite (Plate I fig 9, and Plate II fig 14 and 15). During normal development these host reactions rarely succeed in walling off and checking the growth of the acanthocephalan. In the experiments on the effects of lowered temperatures on development of the acanthocephalan in the amphipod, it was observed that a great many of the larvae (ten days to two months after infection) were walled off and enclosed in a brown chitinous-like covering. Larvae thus covered soon died and degenerated, leaving a hard cyst-like structure attached to the amphipod intestine. Miller (1943) observed similar cyst-like structures in *Popillia japonicum* infected with the developing larva of *Macracanthorhynchus hirudinaceus*.

It appears, then, from the present work that growth of the parasite at the higher temperature is so rapid that the protoplasmic sheath spread over it is being continuously ruptured, preventing hardening from taking place. At the lower temperature growth of the larva is retarded which permits the protoplasmic sheath produced by the amoeboid cells of the host to remain intact for a period of sufficient duration to become hardened.

DISCUSSION

The life cycle of *L. thecatus* under natural conditions shows a seasonal periodicity. In northern areas during the months of May and June the fish host harbors great numbers of immature acanthocephalans of this species. As the season pro-

gresses in summer the number of immature acanthocephalans decreases and the majority present are fully mature males and females. The peak of infection of the fish with *Leptorhynchoides thecatus* occurs during July, August, and September. The number present within the fish remains rather constant during October and November. In this period very few immature individuals are present. During December, January, and February the number of *Leptorhynchoides thecatus* within the fish declines. By the end of February this acanthocephalan is only occasionally found in the fish host. These observations tend to indicate that the length of life of *Leptorhynchoides thecatus* within the fish may be limited to one season.

The question presents itself of how *L. thecatus* overwinters. Is the winter spent in the egg stage, in the amphipod intermediate host, or do both methods serve to carry it through the winter?

In another section of this paper it was pointed out that the eggs of *Leptorhynchoides thecatus* remained viable for nine months stored in the refrigerator at 4° C. This indicates that the eggs of this acanthocephalan are able to survive at low temperatures for extended periods of time.

The effect of temperature on the development of the larva of *L. thecatus* has been considered. At 13° C. the larva of this acanthocephalan required at least two months before it developed to the infective stage within the intermediate host, whereas at 25° C. full development is attained in from thirty to thirty-two days. Although development at temperatures lower than 13° C. has not been studied, it seems logical to conclude that lower temperatures will further decrease the rate of development. The temperature of a lake during the winter months may go as low as 2° C.; thus development of the acanthocephalan larva within the intermediate host is retarded. Because of the lowered temperature of the lake during the winter months, the larvae present within the amphipod intermediate host in the fall of the year are not likely to develop to maturity until the following spring, when the rise in lake temperature occurs.

On the basis of this experimental evidence the author believes that *Leptorhynchoides thecatus* overwinters both as a larva within the amphipod intermediate host and in the egg, free in the water.

In nature, immature forms of *L. thecatus* are found encysted in the mesenteries of a wide variety of fish, including those which are considered the normal hosts of this acanthocephalan.

The probable explanation for the presence of encysted forms of this parasite in the abnormal host is that conditions within the intestine of this fish host are unfavorable for development of the acanthocephalan within the intestinal lumen. The acanthocephalan juveniles swallowed by the abnormal fish host, not being able to establish themselves within the intestine, penetrate through the wall and encyst in the mesenteries (Van Cleave, 1920).

When conducting experimental infection of the fish host by feeding large numbers of infected amphipods, the author observed that the fish contained, besides the Acanthocephala within the intestine, forms encysted within the mesenteries. The fish used in these feeding experiments were hatchery raised and had not been previously exposed to acanthocephalan infection. Control series not fed experimentally contained no Acanthocephala in the intestine or tissues. The encysted larvae present were thus due to the experimental infections.

In an attempt to explain the presence of these forms in the tissues of the normal fish host, juveniles and late acanthella stages of *Leptorhynchoides thecatus* of varying ages were fed to fish known to be ACANTHOCEPHALA-free. From these feedings the following observations were made:

1. Juveniles which were allowed to develop within the amphipod host at least thirty days established themselves within the intestine of the fish host.

2. Young juveniles which had developed within the intermediate host for twenty-six, twenty-seven, and twenty-eight days did not establish themselves within the intestine of the host, but penetrated the wall and encysted in the mesenteries of the host.

3. Late acanthella stages which had developed within the intermediate host for less than twenty-six days were not able to establish themselves within the fish host.

The author believes, on the basis of these experimental results, that forms of *L. thecatus* encysted in the normal host represent larvae not sufficiently mature when ingested by the fish to establish themselves within the intestine.

Further experiments on this phase of the life cycle and on the effect of temperature on development within the amphipod host are at present being conducted.

SUMMARY

1. The life cycle of *Leptorhynchoides thecatus* has been experimentally reproduced in the laboratory.

2. The amphipod, *Hyalella azteca,* serves as the intermediate host, and the rock bass, *Ambloplites rupestris,* as the definitive host.

3. The eggs of *Leptorhynchoides thecatus* are ingested by the amphipod intermediate host. Hatching takes place within the intestine. The acanthor of *Leptorhynchoides thecatus* penetrates the epithelium of the host and locates between it and the serosa of the intestine.

4. The embryo undergoes a period of growth and development attached to the host intestine, producing a rounded bladder-like acanthella projecting into the hemocoele of the host.

5. Fourteen days after entering the intermediate host the acanthella separates from its attachment to the intestine of the amphipod, and enters into a period of development free within the hemocoele of the host.

6. Thirty-two days after the embryo is ingested by the amphipod it has developed into a juvenile acanthocephalan capable of infecting the fish definitive host.

7. The juveniles of *Leptorhynchoides thecatus* within the hemocoele of *Hyalella azteca* contain all the organs of the adult with one exception: the juvenile females do not have the ovary completely formed.

8. Upon ingestion of *Hyalella azteca* by the fish host, the juvenile ACANTHOCEPHALA are released into the fish stomach and attach to the walls of the pyloric cecae.

9. Four weeks after entering the fish host the juvenile male acanthocephalan has completed its development. During this period, the female completes formation of the ovary and eight weeks after entering the fish it passes mature eggs.

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Description of Plates

Plate I

(Value of scale: fig. 1–11, 0.05 mm.; fig. 12-13, 0.02 mm.)

Fig. 1. Mature egg of *Leptorhynchoides thecatus*.

Fig. 2. Acanthor of *Leptorhynchoides thecatus* shortly after being freed of the embryonic membranes within the digestive tract of the amphipod. Note: the anterior larval spines, central nuclear mass, and the giant cortical nuclei.

Fig. 3. Cross section of amphipod intestine showing the acanthor of *L. thecatus* penetrating the epithelium. Note: proliferation of host epithelium and cells collected in the serosa at the site of penetration.

Fig. 4. Cross section of amphipod intestine after penetration is completed showing acanthor located between serosa and epithelium.

Fig. 5. Acanthor two days development within the amphipod. Surface view looking through serosa of the intestine. Note: tissue drawn into anterior pouch-like structure.

Fig. 6. Acanthella three days development. The outward growth of the central portion of the acanthor body and segmentation of the central cellular mass are now quite evident.

Fig. 7. Acanthella four days development in amphipod host. Lateral ventral view. The outward bulge of the acanthor body is now quite pronounced. The giant cortical nuclei are distinct. Central cellular mass has undergone further segmentation.

FIG. 8. Ventral view of same specimen as Fig. 7 removed from tissue. Note: the acanthor spines and lack of rostellum.

FIG. 9. Acanthella five days development within the amphipod. The bulging outgrowth of former acanthor body has become a bladder-like structure projecting into the hemocoele of the amphipod and anchored to the host intestine by the remnant of the acanthor body. The giant cortical nuclei have moved into the periphery of the bladder-like body. The central nuclear mass has undergone proliferation of its elements and moved into the center of the body of the acanthella. Host giant cells have formed a syncytial sheath surrounding the rapidly growing parasite. This protoplasmic sheath is continuous with serosa of host intestine.

FIG. 10. Acanthella six days development. The bladder-like body continues to increase in size. The central nuclear mass has moved entirely within the acanthella body. In this view the remnant of the acanthor body is mechanically twisted.

FIG. 11. Acanthella seven days development within amphipod. Body of acanthella has increased in size. It is beginning to constrict off from the original acanthor body.

FIG. 12. Male acanthella twenty-two days development within amphipod.

FIG. 13. Male acanthella twenty days development within amphipod.

PLATE II

(Value of scale: fig. 15, 16, and 19–0.05 mm.; fig. 14 and 17–0.01 mm.; fig. 18–0.3 mm.)

FIG. 14. Acanthella eight days of development in amphipod host.

FIG. 15. Acanthella nine days of development in amphipod host.

FIG. 16. Acanthella thirteen days of development in the amphipod host. Note: giant cells and protoplasmic processes of host reaction. Central nuclear mass of acanthella has begun to elongate transversely.

FIG. 17. Acanthella fourteen days of development in amphipod host. Note: original acanthor body attached to amphipod intestine and connected to acanthella by a stalk. The central nuclear mass is elongate. The anlage of the proboscis and protoplasmic sheath is visible as a number of large cells at one pole. This establishes the anterior axis of the parasite. At the opposite pole a group of smaller cells form the anlage of portions of the reproductive tract.

FIG. 18. Male acanthella fifteen days of development in the amphipod host. Note: establishment of proboscis sheath, brain, and the anlage of proboscis.

FIG. 19. Female juvenile thirty-two days of development in the amphipod host.

PLATE III

(Value of scale in all figures—0.1 mm.)

FIG. 20–24. Successive stages in the development of the proboscis.

FIG. 20. Twenty day acanthella.

FIG. 21. Twenty-two day acanthella.

FIG. 23. Twenty-three day acanthella.

FIG. 22. Twenty-six day acanthella

FIG. 24. Twenty-nine day acanthella.

FIG. 25. Male acanthella seventeen days development within amphipod host.

PLATE IV

FIG. 26. Female acanthella twenty days development in amphipod host.

FIG. 27. Male acanthella twenty days development in amphipod host.

FIG. 28. Male acanthella twenty-two days development in amphipod host.

FIG. 29. Female acanthella twenty-three days development in amphipod host.

FIG. 30. Male juvenile thirty days development. Note: fragmented nuclei in cortex.

FIG. 31. Male acanthella twenty-nine days development in amphipod host. Note: dendritic nuclei in cortical wall and proboscis hooks piercing the cuticula of the proboscis.

FIG. 32. Female juvenile thirty-two days development in amphipod host. Note: absence of definite ovary.

FIG. 33. Female juvenile one week development in fish host. Note: ovarian mass in genital ligament.

FIG. 34. Young female two weeks development in fish host.

FIG. 35. Young female three weeks development in fish host. Note: ovarian mass has fragmented. Some of ovarian fragments have ruptured into body cavity.

FIG. 36. Young female four weeks development in fish host. Note: proliferation of ovarian substance and body cavity packed with floating ovaries.

FIG. 37. Female six weeks development in fish host. Note: developing embryos are present in body cavity.

FIG. 38. Young male one week development in fish host.

FIG. 39. Male three weeks development in fish host.

FIG. 40. Mature male four weeks development in fish host.

PLATE I

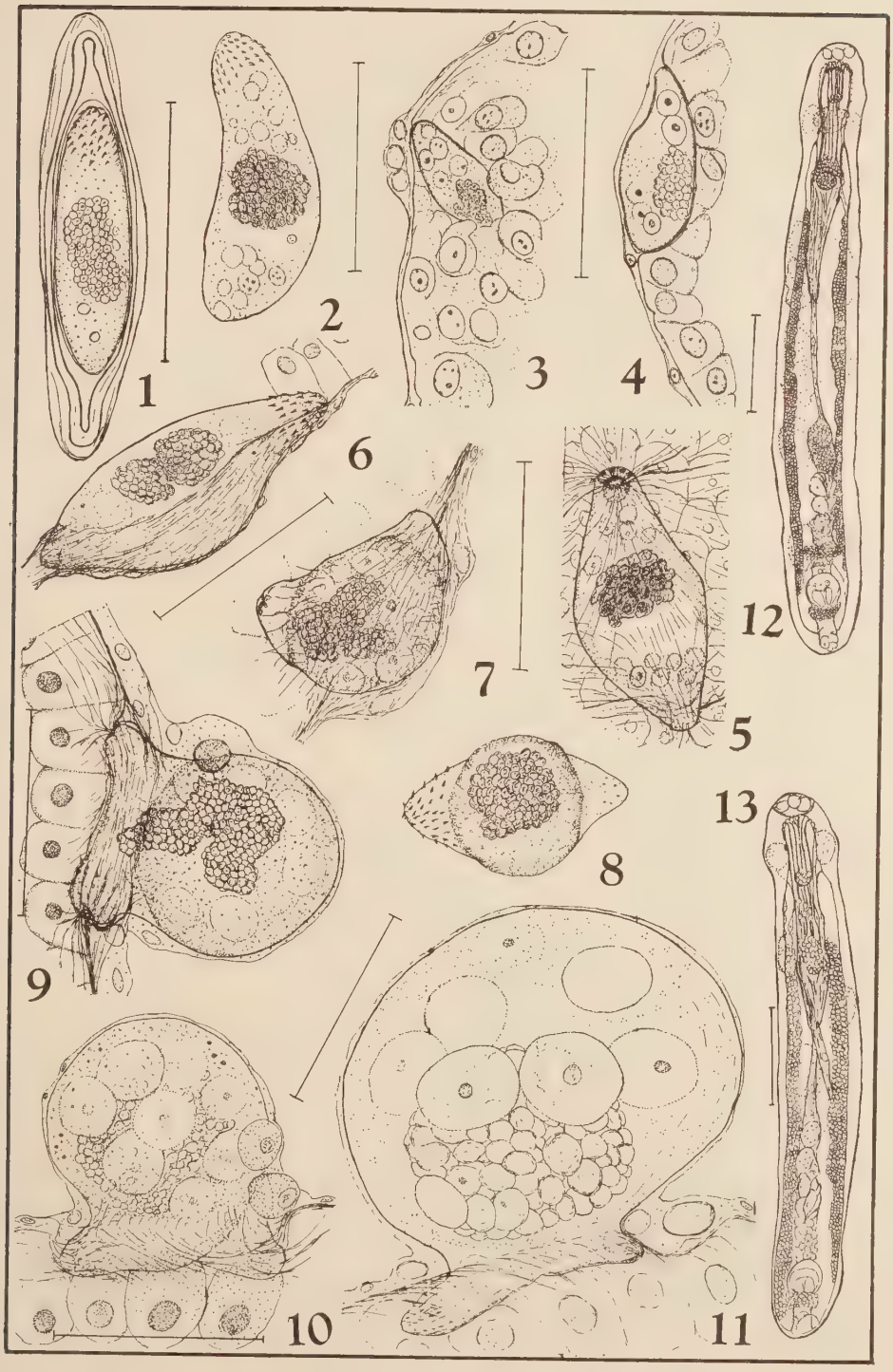


PLATE II

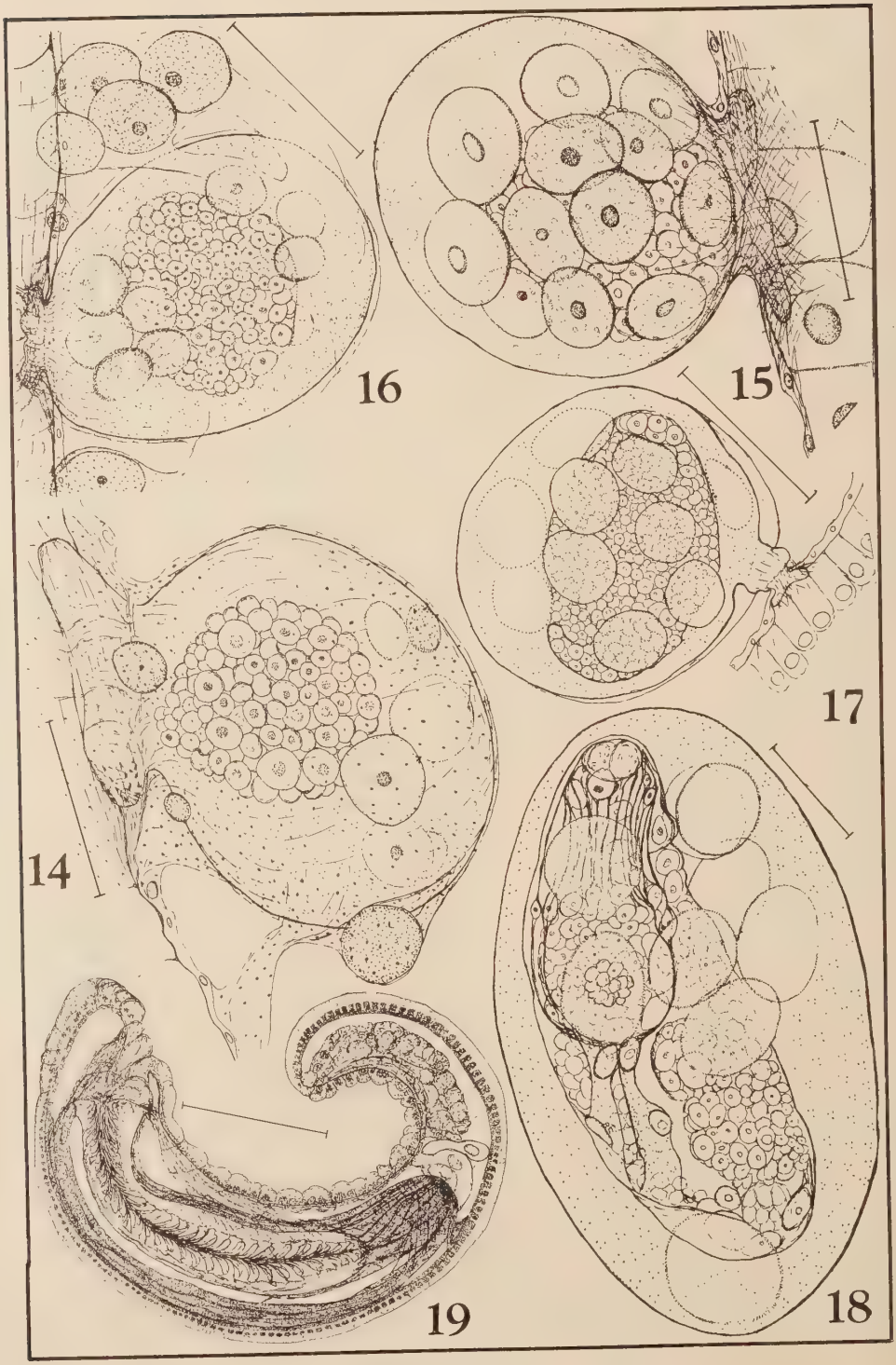


PLATE III

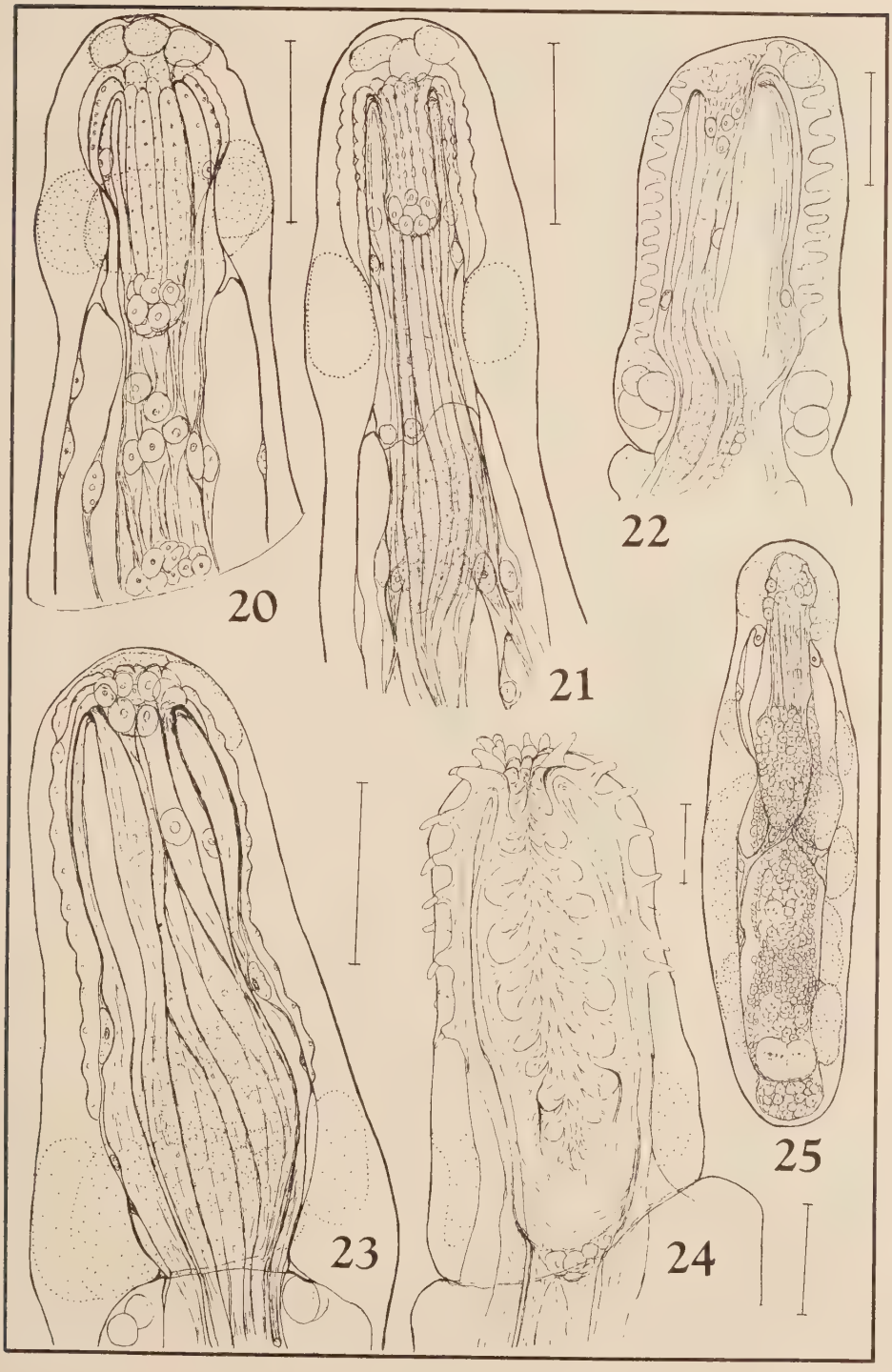
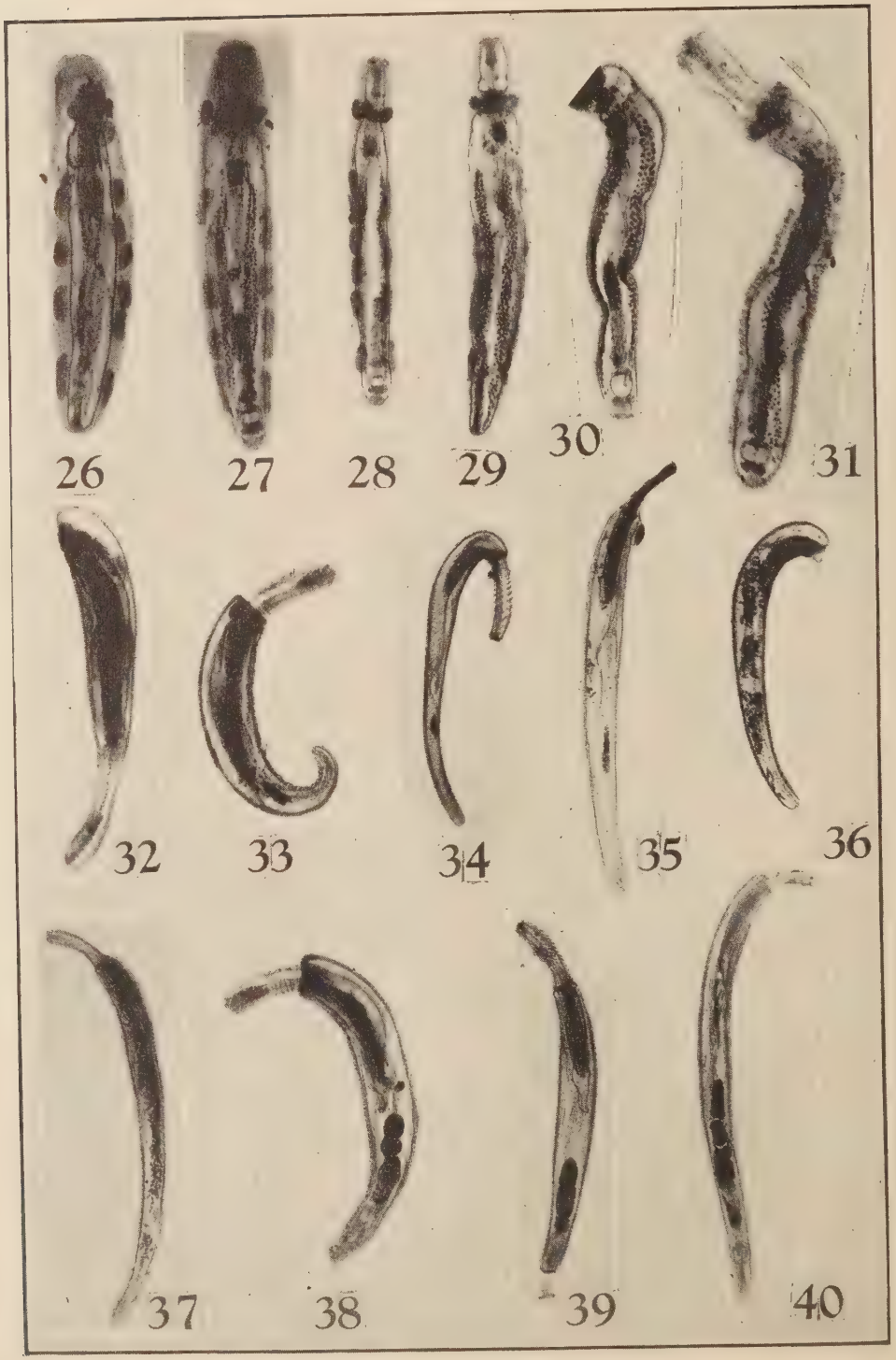


PLATE IV



EXPERIMENTAL STUDIES ON THE SPECIFICITY OF SKIN TESTS FOR THE DIAGNOSIS OF SCHISTOSOMIASIS*

DONALD L. AUGUSTINE AND THOMAS H. WELLER

INTRODUCTION

Cort (1928) demonstrated that certain non-human schistosome cercariae may penetrate the skin of man and cause a dermatitis. This entity, which was termed schistosome dermatitis or swimmer's itch, has now been recognized as a common affliction in many parts of the world. While relatively little is known of the immunological response in man to these cercariae, the evidence indicates that typical schistosome dermatitis does not appear after the primary contact (MacFarlane, 1944; Olivier, 1947), but develops following multiple exposures. MacFarlane, in addition, found that exposed individuals gave a positive intradermal reaction when injected with cercarial antigen. These observations suggest that the non-human schistosome cercariae might be sensitizing agents.

Recently Augustine and Lherisson (1946) have pointed out that certain of the false-positive skin reactions obtained with *Dirofilaria immitis* antigen in suspected cases of human filariasis may be attributable to immune responses produced in man by repeated exposures to the infective filarial larvae of the lower vertebrates. Inasmuch as some schistosomes have been shown to have a common antigenic complex (Fairley and Williams, 1927; Khalil and Hasson, 1932; Katzin and Most, 1946; Wright *et al.* 1947), it would seem that, in a comparable manner, repeated exposures to the dermatitis-producing schistosome cercariae might produce a response which would account for certain of the false-positive immunological reactions encountered in studies on human schistosomiasis. This supposition was raised by Cort (1936) as an explanation for his own sensitivity on intradermal testing with *Schistosoma mansoni* antigen, but apparently this question has not been further investigated. The present paper reports an experimental approach to the question of the immunological relationships between the human and non-human schistosome cercariae. The work was carried out during the summer of 1947 at the University of Michigan Biological Station, Douglas Lake, Michigan.

MATERIALS AND METHODS

Collection and concentration of cercariae. Three species of dermatitis-producing schistosome cercariae, *C. stagnicolae* Talbot, *C. physellae* Talbot, and *C. elvae* Miller, and also one strigeid cercaria, *Diplostomum flexicaudum* Cort and Brooks, were employed in the experiments. The schistosome cercariae are referred to throughout by the names under which they were originally described.[1] Collections of the snail intermediate hosts, *Stagnicola emarginata angulata* (Sowerby), *Physa parkeri* Currier, and *Lymnea stagnalis oppressa* (Say) were made from several locali-

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[1] We wish to express our thanks to Dr. W. W. Cort for his confirmation of the identity of these cercariae.

ties in the Douglas Lake region. Those snails found to be shedding cercariae of the species desired were transferred in the evening to bottles containing 70–100 cc. of well water. The following morning a representative sample of the cercariae in each bottle was examined grossly and microscopically to eliminate the occasional double infection not previously apparent and the dilute cercarial suspensions were then decanted and pooled according to species.

In preliminary experiments attempts were made to concentrate the living cercariae by centrifugation. This method was satisfactory for *D. flexicaudum,* but was only partially successful for the three species of schistosome cercariae. These latter cercariae which were available in comparatively small numbers, tended to disperse rapidly following centrifugation, and the sedimented material contained relatively large amounts of extraneous debris. Previous workers have noted that certain of the dermatitis-producing schistosome cercariae possess positive phototropic and negative geotropic reactions(Cort and Talbot, 1936). These observations suggested that the technique developed by McMullen and Beaver (1945) for the concentration of schistosome miracidia might be applicable in recovering cercariae. Through the use of the light-proofed, side-armed distillation flasks described by McMullen and Beaver, concentrated suspensions of each of the three schistosome cercariae were readily obtained. The water from the bottles containing the night's yield of cercariae was poured in the flask, the water level adjusted, and the exposed portion placed near a light source. Almost immediately cercariae could be seen swimming into the side-arm. The maximum accumulation of the cercariae in the side-arm occurred within one to three hours at which time they could be removed as a concentrated suspension with a pipette. The suspension of cercariae contained no visible debris.

Infection of experimental animals. Six mature rabbits were repeatedly exposed to living cercariae. Applications were carried out by removing the hair from the ventral abdominal and thoracic regions with an Oster animal clipper, and distributing heavy suspensions of cercariae over the skin with a pipette. The animals were then restrained in the supine position until the skin surface was dry. Over a 46-day period, 2 rabbits were exposed 8 times to suspensions of *C. elvae* at intervals ranging from 4 to 11 days. During the same period 4 other rabbits were exposed 8 times, 2 of these receiving applications of *C. stagnicolae,* and 2 of *C. physellae.* Counts of the cercariae were not made, but it was estimated that several hundred were applied at each exposure. Specimens of serum were obtained from each animal prior to the first application of cercariae, and at intervals thereafter, the final specimen being collected 5 weeks after the last exposure.

Preparation of cercarial antigens. Circumstances made it necessary to preserve the daily collections of cercariae during the 8-week period. This was accomplished by mixing the concentrated cercarial suspensions with approximately equal parts of 1% formalin. This material was kept at 5° C. Two weeks after the final collection, the dilute formalin was removed and the sedimented material washed twice with 3 cc. of distilled water. The cercariae were transferred to drying bottles and placed at 5° C. in an evacuated desiccator over anhydrous $CaCl_2$ for 3 days and then left at 37° C. for 6 days. At the end of this time the dried flakes were weighed and ground in an agate mortar for 4 minutes. Sufficient isotonic phosphate buffer, adjusted to a pH of 7.9 with NaOH, was added to the resulting powder to make

an initial dilution of 1:100, 1:500, or 1:1000 depending on the species. The suspensions were left to extract at 5° C. for 7 days, and then centrifuged at 3000 RPM for 10 minutes. Serial doubling dilutions of the various supernatants were prepared in the same buffer solution, bottled in vaccine vials, and used as antigens within a 2-week period as outlined below. The yield of dried cercariae was: *C. physellae,* 9 mg.; *C. stagnicolae,* 10 mg.; *C. elvae,* 3.6 mg.; and *D. flexicaudum,* 49 mg.

In addition to the above, two antigens prepared from cercariae of *Schistosoma mansoni* were available. One was made in Puerto Rico by Dr. José Oliver-Gonzales and supplied by him in the form of a dried powder. This material was ground and extracted in the manner described above and was prepared concurrently with the other antigens. The other antigen was supplied through the courtesy of Drs. W. H. Wright and J. Bozicevich of the National Institutes of Health as a 1:8000 saline extract.

Experimental

The local reaction to application of cercariae. Following the first exposure of the 6 rabbits no cutaneous reaction was observed. The second and third applications of cercariae were administered on the 4th or 5th, and 8th or 9th days respectively, after the original exposure. Twelve hours after each of these exposures, all 6 rabbits showed scattered small petechial lesions from 1 to 2 mm. in diameter. At 24 hours the lesions had developed into confluent pink maculo-papular areas measuring up to 5 mm. in diameter which persisted for another 24 hours and then faded rapidly. The fourth exposure, carried out on the 12th or 13th day after the original application of cercariae, was followed by a more pronounced reaction with the development of a diffuse flaccid edema of the abdominal wall and of a small amount of serous exudate and crusting of the skin. Reactions of this type were seen after all the subsequent exposures.

The abdominal skin of one of the rabbits exposed to *C. stagnicolae* had pigmented areas which were large and well defined. Although the cercariae were evenly distributed, the lesions were few and small within the pigmented patches, but were of usual prominence in the non-pigmented areas. This was a constant occurrence with this rabbit following each application of cercariae.

Intradermal tests

Intradermal tests were performed on 5 of the experimental rabbits and on 2 unexposed control rabbits, between 35 and 38 days after the last exposure to cercariae. The sixth experimental rabbit, exposed to *C. stagnicolae,* was sick and therefore unsatisfactory for skin testing. The tests were carried out by injecting 0.1 cc. of the various antigen dilutions, and of the buffer solution as a control, into the abdominal skin; all of the 17 to 20 dilutions tested on any one animal were applied at one time. The results of these tests are summarized in Table I.

In general, the reactions following the injection of the various antigens were similar. The colorless bleb at the site of inoculation disappeared and was replaced after 15 to 30 minutes by an erythematous area which reached its maximum development in 2 to 3 hours and then gradually faded over a 3-day period; with certain of the antigens the erythematous area showed a varying amount of induration. The reaction to *S. mansoni* (National Institutes of Health) antigen, however, was not of this type, no evidence of erythema or induration being observed.

TABLE I.—*Results of intradermal tests using schistosome and strigeid cercarial antigens on rabbits repeatedly exposed to dermatitis-producing cercariae**

Rabbits exposed to cercariae:	Types of Antigen in Various Dilutions																Buffer control
	C. stagnicolae			*C. physellae*			*C. elvae*			*S. mansoni* (P.R.)†			*S. mansoni* (NIH)‡	*D. flexicaudum*			
	1:1000	1:4000	1:8000	1:1000	1:4000	1:8000	1:1000	1:4000	1:8000	1:1000	1:4000	1:8000	1:8000	1:1000	1:4000	1:8000	
C. physellae #1	11	15	11	5	0	0	15	8	5	10	0	0	0	15	5	10	0
	++	++	++	++	−	−	+	+	+	+	−	−	−	++	++	++	−
C. physellae #2	12	10	9	10	6	3	15	9	7	9	7	0	0	9	6	6	0
	++	++	++	−	−	−	+++	+++	+++	+	−	−	−	++	++	++	−
C. elvae #3	15	14	10	14	11	0	20	14	12	11	9	7	0	13	12	8	0
	++	++	++	++	−	−	+++	+++	+++	+	+	+	−	++	+	−	−
C. elvae #4	13	13	14	10	5	3	16	13	12	10	10	10	0	13	12	12	0
	+++	+++	+++	+	−	−	+++	+++	+++	+	−	−	−	++	++	++	−
C. stagnicolae #5	15	9	8	0	0	0	12	9	4	10	5	0	0	12	12	11	0
	+++	+++	+	−	−	−	+++	++	+	+++	+	−	−	+++	+++	+++	−
Normal unexposed control rabbit #1	15	14	12	12	8	0	18	10	8	8	0	0	0	12	14	12	0
	+++	+++	+++	+	−	−	++	+	+	+	−	−	−	++	++	++	−
Normal unexposed control rabbit #2	10	10	0	0	0	0	0	0	0	0	0	0	0	10	10	0	0
	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−

* Results expressed as average diameter in mm. of reactions 2 to 3 hours after inoculation. Degree of induration graded from — (none) to +++.

† P.R. = Puerto Rico.

‡ N.I.H. = National Institutes of Health.

No definite evidence is shown by the results of intradermal tests that the exposed rabbits developed a specific sensitization. The fact that one control rabbit (No. 1) gave significant reactions to the antigens, suggests that they may have contained a toxic factor. However, control rabbit No. 2 gave negative or minimal reactions. Because of the limited amount of antigen material, more extensive control series could not be tested. It is of interest that both *S. mansoni* antigens gave essentially negative reactions when applied in the dilutions usually employed for skin tests on human beings. It is felt that these results are not conclusive and that further work is indicated because of the theoretical and practical importance of the question.

Precipitin tests

Serum specimens from rabbits infected with each of the three species of cercariae were employed; the specimens from each rabbit included samples taken prior to exposure, and one week and five weeks after the last application of cercariae. Using precipitin tubes with an internal diameter of 2.5 mm., serum specimens were overlayered with doubling dilutions, starting with 1 : 500 and ending at 1 : 32,000, of the antigens prepared from *C. stagnicolae, C. physellae, S. mansoni* (Puerto Rico), and *D. flexicaudum,* and also with *S. mansoni* (National Institutes of Health) in the single dilution of 1 : 8000. In a second experiment, tenfold serum dilutions from 1 : 1 to 1 : 1000 were employed and a constant dilution (1 : 500) of the *C. stagnicolae* and *S. mansoni* (Puerto Rico) antigens were used. All tests were read at 1 hour and at 12 hours. No specific precipitins could be demonstrated.

SUMMARY AND CONCLUSIONS

Rabbits were repeatedly exposed to three species of non-human schistosome cercariae, *C. stagnicolae, C. physellae,* and *C. elvae,* with the production, after the first exposure, of an extensive dermatitis. Approximately seven weeks after the last exposure, intradermal tests were performed on the 5 experimental and 2 control rabbits, using antigens made from the 3 non-human schistosome cercariae, two different antigens prepared from cercariae of *Schistosoma mansoni,* and an antigen made from cercariae of a strigeid, *D. flexicaudum.* Essentially negative reactions were obtained with the two *Schistosoma mansoni* antigens when employed in high dilutions. The results obtained with the other antigens were equivocal.

An effective method for the concentration of living dermatitis-producing schistosome cercariae was developed by utilizing the positive phototropic and negative geotropic reactions of the cercariae.

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TRACHEATION IN CHIGGERS WITH SPECIAL REFERENCE TO *ACOMATACARUS ARIZONENSIS* EWING (ACARINA, TROMBICULIDAE).[1]

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Tracheae and spiracles are not common to all larval TROMBICULIDAE; in fact, their presence is the exception rather than the rule. In mites, in the absence of a tracheal system, one may presume a situation analogous to that of the atracheate insects in which respiration is accomplished by gas diffusion through the body integument.

Tracheae and spiracles have been observed by the author and by others in the two monotypic genera, *Apolonia* Torres and Braga 1939 and *Womersia* Wharton 1947 of the subfamily APOLONIINAE and in the genera *Leeuwenhoekia* Oudemans 1911, *Acomatacarus* Ewing 1942, *Hannemania* Oudemans 1911, *Odontacarus* Ewing 1929 and *Whartonia* Ewing 1944 of the subfamily LEEUWENHOEKIINAE comprising about 50 species. However, in the descriptions of *Whartonia vellae* (Dumbleton) and *Acomatacarus lygosomae* Dumbleton, it is stated that neither spiracles nor tracheae could be discerned. They have not been observed in *Comatacarus* Ewing 1942 and *Chatia* Brennan 1946 of the LEEUWENHOEKIINAE nor in the subfamilies WALCHIINAE and TROMBICULINAE, in the aggregate, about 300 species.

Kawamura (1926, p. 198) in his description of the internal anatomy of the larva of presumably *Trombicula akamushi* Brumpt, which has no tracheal system, misinterpreted the urstigma (a heavily sclerotized pit-like structure of unknown function associated with the posterior distal angle of coxa I) as a spiracle from the "atrial cavity" of which he proceeded to trace the "main tracheal trunk." While the entire account is confused and misleading, at one point it is apparent that Dr. Kawamura is referrring to the sclerotized processes within the gnathosoma as the supposed "primorida of the air chambers of the respiratory organ."

Wharton (1946, p. 157)in his extensive observations on *Ascoschöngastia indica* (Hirst), another species lacking a tracheal system, has suggested that, of the two paired skeletal structures within the body of the gnathosoma, the medial pair of tube-like structures may represent sclerotized trunks of the respiratory system since they are in the position of the tracheal system of later stages of related non-trombiculid mites.

The tracheal system in trombiculid larvae was mentioned by Womersley (1944, p. 82) in several species of *Acomatacarus* which he referred to the genus *Leeuwenhoekia*. The importance of the spiracles and tracheae as taxonomic characters prompted him to erect a new subfamily, LEEUWENHOEKIINAE, which he later raised to familial status, LEEUWENHOEKIIDAE (Womersley, 1945, p. 96).

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[1] From the Rocky Mountain Laboratory (Hamilton, Montana), Microbiological Institute, National Institutes of Health.

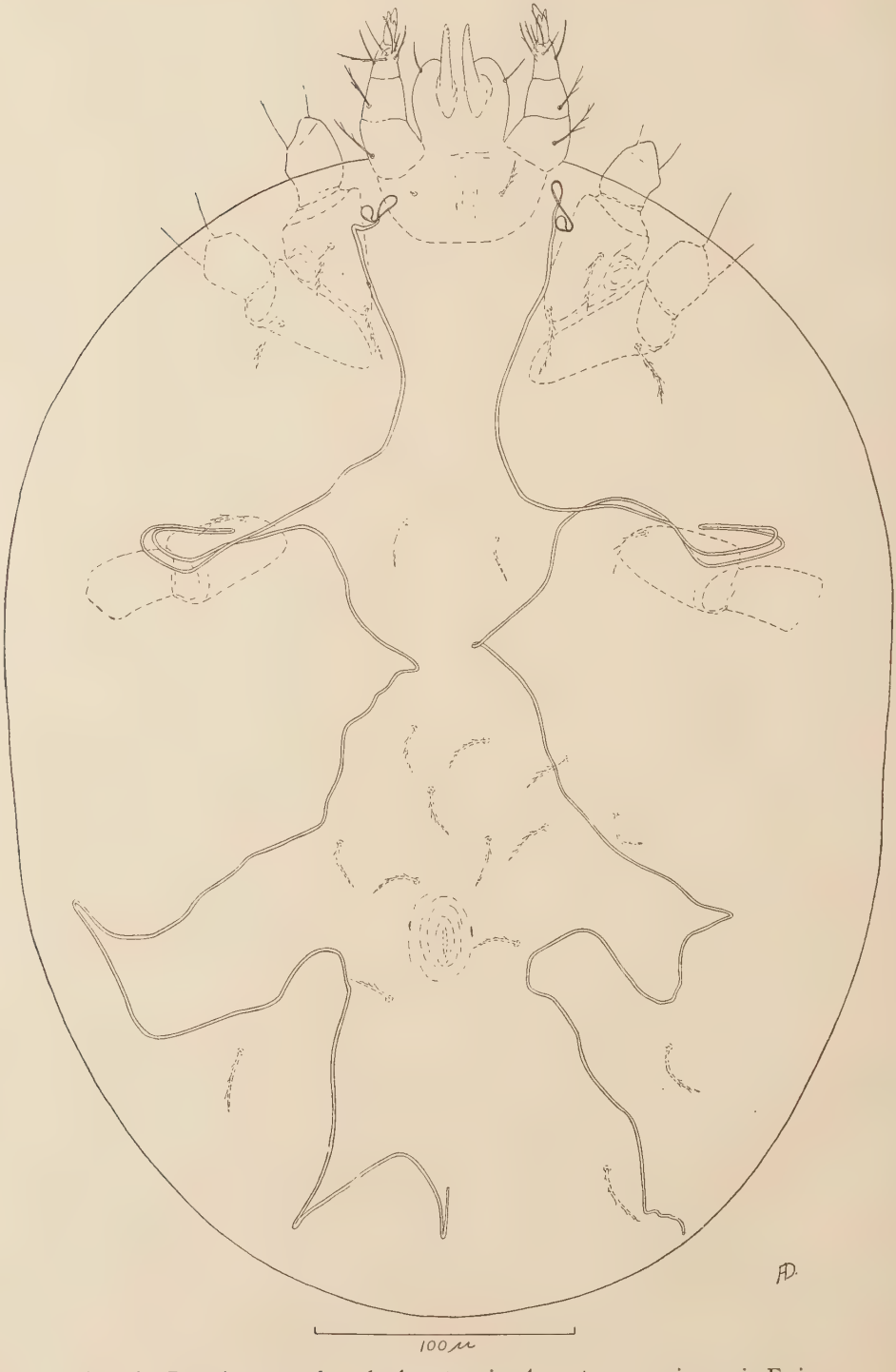

FIG. 1. Dorsal aspect of tracheal system in *Acomatacarus arizonensis* Ewing.

FIG. 2. Spiracle and anterior portion of trachea in *Acomatacarus arizonensis* Ewing showing the narrow, elongate neck connecting the atrium with the main tracheal trunk; the taenidia; the characteristic convolutions of the trachea in the gnathosomal area and region of coxa III. Dorsal view.

In a collection of chiggers recently received from Dr. J. K. Frenkel was a large series of *Acomatacarus arizonensis* Ewing from the lizards *Phrynosoma coronatum blainvilli* Gray and *Sceloporus magister magister* Hallowell collected at Walker Pass, California. The parasites had been preserved in Hollande's cupric-picro-formol-acetic fixative for sectioning and are of particular interest because of the relative ease with which the tracheal system can be traced.

In *Acomatacarus arizonensis,* as in all tracheate larval TROMBICULIDAE, a spiracle (fig. 1) is located on each side of the venter between coxa I and the base of the gnathosoma, its relative position varying somewhat with the degree of engorgement of the larva. The spiracle is funnel-shaped (fig. 2) and moderately sclerotized. Its diameter at the orifice, in 49 specimens measured, varies from 3.02 to 5.20 microns and averages 4.35 microns.

Both Womersley (1945, p. 96) and Wharton (1947, p. 382) indicate the branching of tracheae. In the 51 specimens of the series of *A. arizonensis* studied a single, unbranched trachea (fig. 1) arises from the atrium of the spiracle and continues in a tortuous course to the posterior end of the body where it terminates abruptly without closing. At its origin, and for a short distance beyond, the trachea is very narrow, sometimes barely within the limits of resolution of the microscope. The diameter then increases rather suddenly, then very gradually decreases posteriorly to the end. Throughout the greater portion of the trachea, spirals of cuticular intima, the taenidia, are visible (fig. 2). Each trachea greatly exceeds the length of the body. In 48 specimens measured, the diameter of the trachea at approximately the widest point varies from 1.56 to 2.60 microns and averages 1.88 microns. The several convolutions of the trachea in the immediate vicinity of the spiracle and gnathosomal area and the bending and folding back on itself in the region of coxa III appear to be characteristic for the species.

In *Whartonia perplexa* (Brennan) the system is similar up to the point at which the trachea bends toward coxa III where it becomes lost in a mass of fat tissue. The very narrow, elongate neck leading from the atrium into the trachea proper and the series of convolutions in the gnathosomal area are apparent. In *W. nudosetosa* (Wharton), again the narrow neck and convolutions near the spiracle, followed by the relative straightening of the trachea in its posterior course, are readily seen. There is no evidence of the branching of the trachea in either species.

Since in morphologic studies it is not often possible to trace the complete tracheal system in a trombiculid larva, and since the additional supporting observations are but fragmentary, it becomes largely a matter of speculation whether or not the pattern described for *A. arizonensis* is representative of other tracheate species.

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Author's Note

After the above paper had gone to press and it was too late to alter the text by incorporating additional material, two more articles dealing with tracheation in chiggers became available to me.

André, 1943, described and figured the tracheal system of the larva of *Acomatacarus paradoxa* (André), a species parasitic on scorpions and erroneously placed in the genus *Leeuwenhoekia*, while Hoffmann, 1948, has described and figured the same for *A. chiapanensis* Hoffmann. The course taken by the tracheae in the former species is very similar to that in *A. arizonensis* Ewing, while in the latter species each trachea performs innumerable convolutions in its course through the body. In neither species are the tracheae branched.

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James M. Brennan
9 May 1949

OBSERVATIONS ON *PHYLLODISTOMUM LOHRENZI* (LOEWEN, 1935), (TREMATODA: GORGODERIDAE).[1]

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There is apparently considerable variation in the morphology of *Phyllodistomum lohrenzi* (Loewen, 1935). The present paper records morphological variations from the original description found in specimens collected at Grand Rapids, Ohio. These specimens agree generally with the material reported by Venard (1940), but certain observations made have not been previously reported.

Observations have been made on 40 whole mounts and three serially sectioned specimens collected from centrarchid fishes by Dr. E. E. Dickerman and the writer during the summers of 1944–1946. Specimens were loaned for comparison by Drs. Loewen, Venard, and Byrd.

Collections were made from *Lepomis cyanellus* (Raf.), the green sunfish, and *L. gibbosus* (L.), the pumpkinseed, from the Maumee River. (Host names are based on Hubbs and Lagler, 1947). The writer has found 17.2% of 116 centrarchid fishes infested. Dickerman's collections were made during the summers of 1944 and 1945. The writer's collections were made between July 9 and August 1, 1946. Examination of six fishes between August 1 and October 19, 1946, yielded no additional specimens.

While Loewen (1935) reported a "dull, reddish-brown color" and Venard (1940) reported a "light yellow or cream" color, examination of living worms showed all gradations from creamy white in the smallest and most immature specimens, to a deep reddish-brown in the largest and therefore probably oldest specimens.

Fixed specimens from the Grand Rapids collection measured 1.09 mm. to 6.00 mm. in length by 0.51 mm. to 2.55 mm. in width, both smaller and larger than Loewen's and Venard's fixed specimens.

Two monorchid specimens were observed, the anterior testis being absent from two large, probably old, specimens. In another large specimen the anterior testis was decidedly atrophied. A possible relationship between senescence and degeneration of the testes is suggested. Venard (1940) has mentioned degeneration of gonads, but does not state whether testes, ovaries or both are involved.

Of the 40 specimens collected by Dickerman and the writer, 35 showed oral suckers larger than their ventral suckers, three showed 1.00 : 1.00 ratios, and two specimens showed a ratio of 1.00 : 1.13. The latter five specimens showed evidence of contraction in fixation, while the other 35 were well-relaxed. The range of ratios for the 40 specimens was 1.64 : 1.00 to 1.00 : 1.13 (av. ratio 1.13 : 1.00).

The genital complex (Figure 1.) of *P. lohrenzi* has been examined in serial sections, and the paths of its ducts determined. Inasmuch as there are characteristics which differ from those described for certain other phyllodistomes, it seems ap-

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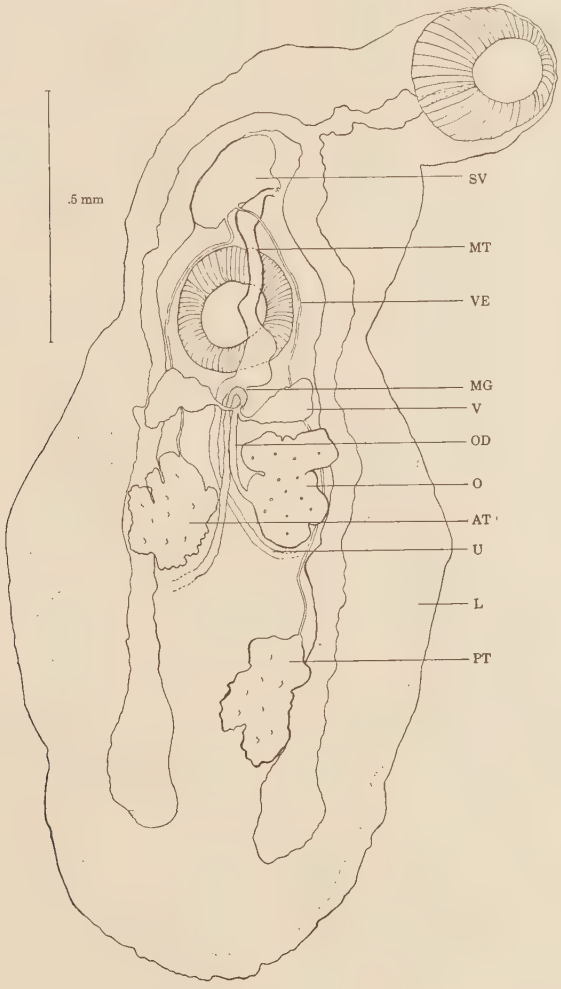
[1]Studies leading to this report were begun with Dr. E. E. Dickerman, Bowling Green State University, and concluded with Dr. G. W. Wharton, Duke University. Drs. S. L. Loewen, C. E. Venard, and E. E. Byrd have loaned specimens for the studies. Their cooperation is appreciated.

propriate that the system should be described here. (Cf. Arnold, 1934; Fischthal, 1942 & 1943; Goodchild, 1943; Groves, 1945; Loewen, 1929; Rankin, 1937; Steelman, 1938; Wu, 1938; Yamaguti, 1934.) The oviduct originates near the posterior medial angle of the ovary and turns antero-medially to a point beyond the anterior level of the ovary. At this point it receives the vitelline ducts and continues for-



EXPLANATION OF PLATE

Fig 1. *Phyllodistomum lohrenzi* (Loewen, 1935). AT—Anterior testis; L—Limit of uterine coils; MG—Mehlis' gland; MT—Metraterm; O—Ovary; OD—Oviduct; PT—Posterior testis; SV—Seminal vesicle; U—Uterus; V—Vitellarium; VE—Vas efferens.

ward where a gland of Mehlis surrounds the oötype. From the oötype the uterus turns ventro-posteriorly following a much convoluted path which occupies all the extra- and inter-cecal space posterior to the vitellaria which is not occupied by other organs. The uterus takes a medial course cephalad, passing ventral to the oötype, thence dorsal to the ventral sucker. A metraterm leads to a genital atrium which opens through a ventral genital pore. Laurer's canal was not observed. A vas

efferens leaves the anterior margin of each testis and courses cephalad ventral to the ovary and vitellaria. The vasa efferentia enter the posterior-lateral margins of a well-developed seminal vesicle that extends anterior to the genital pore. The seminal vesicle communicates with the genital atrium adjacent to the opening of the metraterm.

SUMMARY AND CONCLUSIONS

1. *Phyllodistomum lohrenzi* (Loewen, 1935) is reported from *Lepomis cyanellus, L.humilis,* and *L. gibbosus* taken from the Maumee River at Grand Rapids, Ohio.

2. Observed morphological differences are recorded. It is suggested that the color of living specimens is related to the state of maturity of the worm.

3. The courses of the reproductive ducts in *P. lohrenzi* have been established by the study of serial sections.

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INFLUENCE OF SOME POTENTIAL MOLLUSCACIDES ON THE OXYGEN CONSUMPTION OF *AUSTRALORBIS GLABRATUS*

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The killing mechanisms of chemical compounds used in the control of harmful or obnoxious invertebrates are quite variable. The problem has been investigated to some extent for the case of insects, but no data whatever seem available for snails in general and the trematode-transmitting species specifically.

The urgent need for new molluscacides, more potent than the much applied copper sulfate or similar substances, has led to a survey of the snail-killing properties of a variety of chemical compounds, the results of which will be reported elsewhere by Nolan and Mann. During this work a number of potential molluscacides was uncovered. These, as well as some inactive compounds, were studied by us in respect to their inhibitory effect on the oxygen consumption of *Australorbis glabratus,* the intermediate host of *Schistosoma mansoni* in the West Indies and South America.[1]

The investigation of this phase was singled out for two reasons. On the one hand, the normal aerobic respiration of *Australorbis* has been studied previously (von Brand, Nolan, and Mann, 1948), thus giving a starting point for the study of the pathological respiratory physiology. Secondly, previous studies have shown that some of the compounds employed, notably some naphthoquinones, inhibit enzyme systems involved in the oxygen consumption or glycolysis of various invertebrates (Wendel, 1946; Bueding, Peters, and Waite, 1947; Hoffman-Ostenhof, 1947), as well as of vertebrates (Gemmill, 1948). In a primarily aerobic organism, like *Australorbis,* the oxygen consumption is one of the best indicators of the overall metabolic activities. It was therefore hoped that a study of this kind would furnish leads as to whether the mechanism by which snails are killed involves the inhibition of metabolic enzymes.

MATERIAL AND METHODS

Laboratory-reared *Australorbis glabratus* from Venezuelan stock, weighing individually between 200 and 400 mg., served as experimental animals. Prior to the actual determinations, they were wiped dry with filter paper and weighed to the nearest mg. in groups of two on an analytical balance.

Two snails were then introduced into each Warburg flask. The flasks had a total capacity of about 16 cc.; 2 cc. of medium were employed and the CO_2 was absorbed in the usual manner by means of 10 percent KOH. The temperature of the water bath was 30° C. and the manometers were shaken 100 times per minute with an amplitude of 4 cm.

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[1] All the naphthoquinones and phenanthrenequinones used were prepared in Dr. L. F. Fieser's laboratory and received either directly from him or from Dr. E. Bueding to both of whom we are greatly indebted. The other compounds were received from various sources which will be acknowledged in the forthcoming paper by Nolan and Mann.

Five to 6 individual experiments were carried out with each compound. The normal respiratory rate of the snails was first established in dechlorinated tap water by taking 4 readings at 15-minute intervals. This control medium was then replaced by 2 cc. of dechlorinated tap water containing the test compound at a specified concentration. After reequilibration to the temperature of the water bath, readings were taken for 4 hours, usually during the first hour of exposure at 15-minute intervals and at 30-minute intervals during the remaining time. In cases of pronounced respiratory inhibition the snails were then placed in beakers containing about 200 cc. dechlorinated tap water which was aerated by a slow stream of air. The following morning their respiratory rate was again determined in dechlorinated tap water in order to study whether a recovery from the influence of the poison had taken place.

The procedure outlined above was used for the introduction of the test compound in preference to the tipping in from a side arm in order to avoid any effect from a possible volatility of some compounds during the initial period. The compounds were usually made up as 1 or 0.5 percent stock solutions in acetone or alcohol which were then appropriately diluted with dechlorinated tap water. The solvents, in the dilutions employed, had no effect on the snails.

RESULTS

The various compounds employed are listed in table 1 which shows also their concentration both in terms of molarity and in p.p.m. This latter expression has been added because it is in common use in actual control measures. The former, obviously, is of greater significance for physiological considerations. In the following column of table 1 the type of response to the compounds is specified; typical examples are shown in more detail in Figure 1 which illustrate the rate of respiration during exposure in percent of the pre-exposure values.

It is evident that 4 types of response could be distinguished. In type 1 no distinct influence of the compounds on the rate of oxygen consumption occurred. The variations between the different hours of exposure were more or less variable. The average oxygen consumption over the entire period of exposure (table 1) was not significantly different from the pre-exposure value.

In type 2 a more or less pronounced inhibition occurred initially, ranging with the different compounds anywhere from 15 to 99 percent. During the later stages of the experiments, however, the respiratory rate of the snails rose again in varying degrees. The values obtained during the fourth hour varied from 23 to 110 percent of the pre-exposure values. In some cases, as with 2-chloro-1,2-naphthoquinone (Survey No. 347, Fig. 1) the rise was spectacular. Obviously, with this type of response no average inhibition figure could be computed.

The compounds giving response type 3 showed a relatively slow inhibitory action. An approximately steady level of oxygen consumption was reached in some instances from the second hour on; in others it was not yet reached at the end of the 4-hour exposure period. The inhibition figures recorded in table 1 are, in the former case, the averages of the figures obtained during the steady state, and, in the latter, the figures found during the fourth hour. They probably do not represent the maximal inhibition that would have been observed during longer exposure.

Type 4, finally, represents the reaction to compounds reaching their maximal effectiveness rapidly; that is, during the equilibration period. The inhibition figures

(table 1) are derived from the entire exposure period. In almost all cases showing this type of response the recovery values were also determined. They were in all cases higher than those obtained during the period of exposure but lower than the pre-exposure values ranging, with one exception, between 54 percent and 98 percent of the pre-exposure values. This, however, does not necessarily indicate a longer lasting after-effect of the compounds. It has been shown previously (von

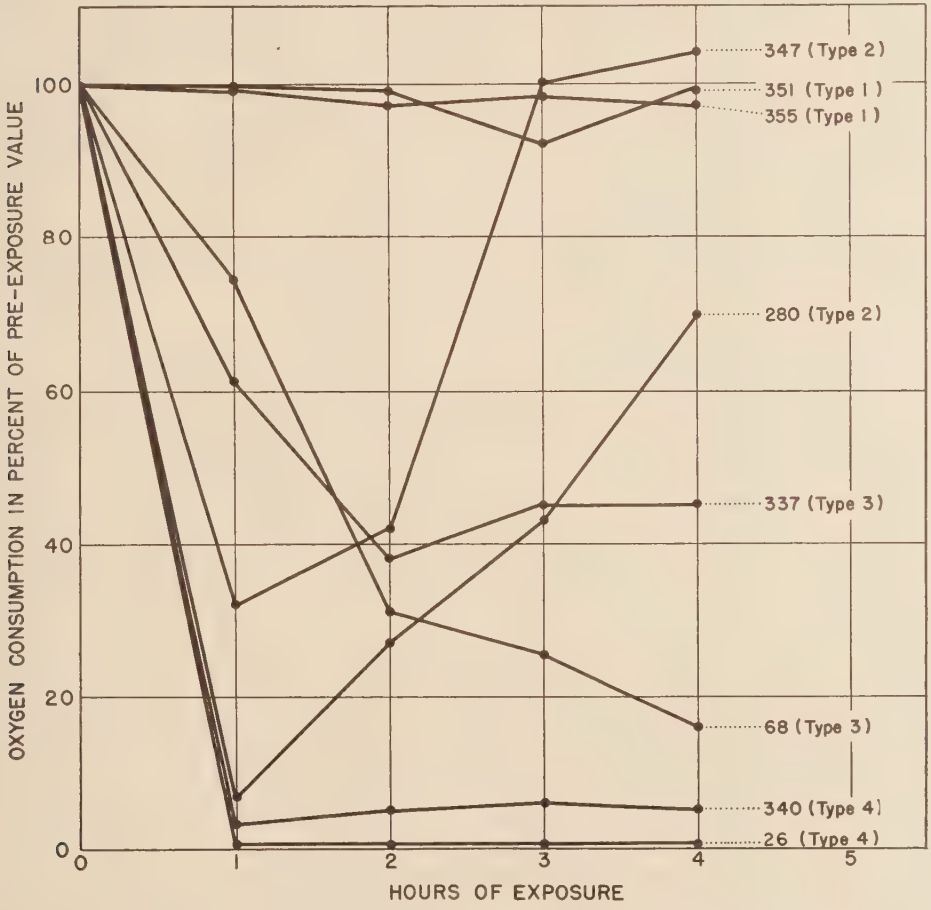

FIG. 1. Oxygen consumption of *Australorbis glabratus* under the influence of dilute solutions of some potential molluscacides. Each point represents the mean value of 6 determinations. The compounds can be identified by means of their survey number and reference to table 1.

Brand, Nolan, and Mann, 1948) that even short periods of starvation reduce very rapidly the rate of oxygen consumption of normal *Australorbis* specimens. Most of the observed recovery values can be explained by the fact that the snails had then starved for 24 hours. The tentative assumption of complete recovery seems justified. A definitely abnormally low recovery value (38 percent of the pre-exposure value) was observed only in the case of α-nitrostilbene (Survey No. 26) which was also the most effective inhibitor.

TABLE 1.—*List of compounds*

Survey number	Name of compound	Concentration		Type of reaction	Average inhibition (−) or stimulation (+) of oxygen consumption in percent of pre-exposure value
		Molar ($M \times 10^{-5}$)	p.p.m.		
	I. Aliphatic compounds				
	A. Carbon dioxide derivatives				
269	1-Dodecylguanidinehydrobromide	5.8	17.9	3	−92 ± 2.2
	II. Carbocyclic compounds				
	A. Cyclohexanes				
272	Δ-1,2,3,4,5,6,-Hexachlorocyclohexane	5.8	16.9	4	−71 ± 2.2
19	*N*-Cyclohexyl-2,3,4,6,-tetrachlorophenoxy-acetamide	2.7	10.0	3	−75 ± 4.1
355	Camphorquinone	5.8	9.7	1	− 3 ± 7.4
	B. Benzene derivatives with 1 benzene ring				
	1. Hydrocarbons				
186	1-Bromo-2,4-dinitrobenzene	4.0	10.0	4	−85 ± 1.7
293	Acetic acid, benzenethiol ester	5.8	8.8	1	−20 ± 12.4
294	*o*-Ethyl-benzenethiol, acetate	5.8	10.5	1	+ 3 ± 23.4
158	2-Pivalyl-1,3-indandione	4.3	10.0	1	−13 ± 10.5
	2. Phenols				
285	1-(2-Chloroallyloxy)-2-toloxy-ethane	5.8	13.1	2	
138	2-Chloro-6-phenylphenol	5.8	11.9	2	
180	4-Chloro-6-phenylphenol	4.9	10.0	2	
280	2-Bromo-4-phenylphenol	5.8	14.4	2	
18	2-*sec*-Butyldinitrophenol	4.2	10.0	4	−82 ± 1.2
17	2-Cyclohexyl-4,6-dinitrophenol, dicyclohexylamine salt	2.2	10.0	4	−79 ± 2.5
295	Quinone	9.2	10.0	3	−96 ± 0.9
296	Hydroquinone	9.1	10.0	3	−94 ± 2.0
302	2-Bromo-1,4-hydroquinone	5.8	10.9	1	−10 ± 9.1
366	2,6-Xylohydroquinone, *bis* (methylcarbamate)	5.8	14.6	1	+14 ± 3.5
365	Thymohydroquinone, *bis* (methylcarbamate)	5.8	16.3	2	
354	2,6-Dimethoxyquinone	5.8	9.8	2	
161	Tolu-*p*-quinone	5.8	7.1	2	
	C. Benzene derivatives with 2 or more benzene rings				
	1. Diphenyl group				
26	*a*-Nitrostilbene	4.4	10.0	4	−99 ± 0.9
	2. Naphthalene group				
343	1,2-Naphthoquinone	5.8	9.2	2	
346	6-Hydroxy-1,2-naphthoquinone	5.8	10.2	4	−81 ± 1.1
347	3-Chloro-1,2-naphthoquinone	5.8	11.2	2	
350	4-Amino-1,2-naphthoquinone	5.8	10.0	1	+ 5 ± 18.1
328	7-Methoxy-1,2-naphthoquinone	5.8	10.9	4	−86 ± 1.9
351	Potassium 1,2-naphthoquinone-4-sulfonate	5.8	16.0	1	− 3 ± 4.7
297	1,4-Naphthoquinone	6.3	10.0	4	−97 ± 0.2
342	2-Methyl-3-hydroxy-1,4-naphthoquinone	5.8	10.9	2	
206	2-Hydroxy-3-isoamyl-1,4-naphthoquinone	4.1	10.0	2	
207	2-Hydroxy-3-pentadecyl-1,4-naphthoquinone	2.6	10.0	3	−30 ± 2.4
208	2-(4-Cyclohexylbutyl)-3-hydroxy-1,4-naphthoquinone	3.2	10.0	4	−86 ± 1.5
210	2-[3(*p*-Chlorophenyl)propyl]-3-hydroxy-1,4-naphthoquinone	3.1	10.0	4	−85 ± 2.4
313	2-Bromo-1,4-naphthoquinone	5.8	13.7	2	
325	6-Chloro-1,4-naphthoquinone	5.8	11.2	4	−97 ± 0.6
44	2-Methyl-1,4-naphthoquinone	5.8	10.0	4	−95 ± 1.3
345	2-Methyl-6-acetyl-1,4-naphthoquinone	5.8	12.4	4	−92 ± 1.5
309	2-Methyl-6-propionyl-1,4-naphthoquinone	5.8	13.2	4	−95 ± 1.7
310	2-Methyl-6-*n*-butyl-1,4-naphthoquinone	5.8	14.1	4	−97 ± 0.5
334	2-Methyl-6-*n*-caproyl-1,4-naphthoquinone	5.8	15.7	4	−75 ± 7.0
311	2-Methyl-6-succinyl-1,4-naphthoquinone	5.8	15.8	2	
298	2-Methyl-3-methoxy-1,4-naphthoquinone	4.9	10.0	3	−24 ± 12.5
326	2-Methyl-6-carboxy-1,4-naphthoquinone	5.8	12.5	1	−19 ± 11.3
327	2-Methyl-3-*n*-thiopropyl-1,4-naphthoquinone	5.8	14.3	2	
344	2-Methyl-3-isothiopropyl-1,4-naphthoquinone	5.8	14.3	2	
332	2-Methyl-3-*n*-thiobutyl-1,4-naphthoquinone	5.8	15.1	2	
333	2-Methyl-3-thioethyl-6-butyryl-1,4-naphthoquinone	5.8	17.5	4	−90 ± 2.7
340	2-Thioethyl-1,4-naphthoquinone	5.8	12.7	4	−95 ± 1.1
336	2-Isothiopropyl-1,4-naphthoquinone	5.8	13.5	4	−97 ± 0.7
338	2,3-Dithiopropyl-1,4-naphthoquinone	5.8	17.8	4	−95 ± 1.3
337	2-*n*-Thioamyl-1,4-naphthoquinone	5.8	15.1	3	−55 ± 10.0
341	2,3-Dithiobenzyl-1,4-naphthoquinone	5.8	23.3	2	
330	2-Thiophenyl-1,4-naphthoquinone	5.8	15.4	4	−85 ± 2.7

TABLE 1 (*Continued*)

Survey number	Name of compound	Concentration		Type of reaction	Average inhibition (−) or stimulation (+) of oxygen consumption in percent of pre-exposure value
		Molar ($M \times 10^{-5}$)	p.p.m.		
329	2(2′-Methylthiophenyl)-1,4-naphthoquinone	5.8	16.3	4	− 85 ± 2.0
307	Potassium 1,4-naphthoquinone-2-sulfonate, monohydrate	5.8	17.1	1	− 13 ± 3.7
331	10-(1,4-Naphthoquinonyl-2)-decanoic acid	5.8	19.0	4	− 89 ± 2.0
335	Methyl-10-(1,4-naphthoquinonyl-)-decanoate	5.8	19.9	4	− 55 ± 9.8
314	Methyl-*S*-(2-methylnaphthoquinonyl-3)-thioacetate	5.8	16.0	2	
	3. Anthracene group				
358	Anthraquinone	5.8	12.1	2	
359	2-Methylanthraquinone	5.8	12.9	2	
211	1-Acetylanthraquinone	5.8	14.5	2	
209	Tetrahydroanthraquinone	5.8	12.3	3	− 54 ± 5.1
	4. Phenanthrene group				
312	Phenanthrenequinone	5.8	12.1	4	− 95 ± 0.4
387	2-Hydroxy-9,10-phenanthrenequinone	5.8	13.0	4	− 95 ± 1.0
388	2-Amino-9,10-phenanthrenequinone	5.8	13.0	4	− 91 ± 0.8
339	Methylphenanthrenequinone-3-carboxylate	5.8	15.2	2	
	5. Cyanine group				
168	[2,5-Dimethyl-1-phenylpyrrole-(3)][1,7-dimethylquinoline-(2)]dimethinecyaninechloride	2.6	10.0	3	− 75 ± 6.2
166	[2,5-Dimethyl-1-phenylpyrrole-(3)][8-chloro-1-methylquinoline-(2)-] dimethinecyaninechloride	2.6	10.6	4	− 59 ± 8.5
61	[2,5-Dimethyl-1-phenylpyrrole-(3)][1-methylquinoline-(4)]dimethinecyaninechloride	2.6	9.5	1	+ 6 ± 22.2
	III. Heterocyclic compounds				
	A. Quinolines				
68	2-β-Anilinovinyl-6-methylquinolinemethochloride	3.2	10.0	3	− 84 ± 4.6
	B. Quinoliniums				
167	1-(2-Ethoxyethyl)-6-methyl-2-[-2,5-dimethyl-1-phenyl-3-pyrryl) vinyl]quinoliniumchloride	2.6	10.0	3	− 92 ± 3.7

DISCUSSION

It is obvious that any physiological activity due to compounds of types 1 and 2 cannot have its basis in an interference with the respiratory enzyme chain of the snail. The initial inhibition observed in type 2 is probably due to the sharp initial retraction of the animals into their shells which prevents the uptake of oxygen into the lung from the gas phase of the flask and which reduces markedly the tissue surface in contact with water through which dissolved oxygen can enter the body. It was often noticed that with this type of compound the snails expanded again more or less towards the end of the experiments.

It is unfortunate that no control experiments could be devised by which snails could have been forced back into their shells and kept in varying degrees of retraction throughout an experiment. Such experiments would have facilitated the interpretation of reaction types 3 and 4. Both types may be due to true respiratory inhibition, that is, to an interference with the respiratory enzymes. The slower reaction observed in type 3 as against type 4 could be due to slower penetration of the compounds. The assumption of true respiratory inhibition seems justified in the case of α-nitrostilbene which inhibited the oxygen consumption by more than 99 percent. It seems very unlikely that such an extreme and lasting inhibition could have been solely due to a retraction. One has probably to assume that the snails survived the 4-hour exposure period by going over to a primarily anaerobic type of metabolism. With the other

substances belonging to types 3 and 4, the assumption of true respiratory inhibition must remain tentative and clarification must await work involving different techniques; for example, the use of homogenates. It does find support, however, in some cases at least by analogy with other invertebrates. It has been mentioned in the introduction that the naphthoquinones act in other cases *via* enzyme inhibition.

We have too few representatives of most chemical groups of compounds to warrant definite statements concerning the question of possible connections between chemical constitution and physiological activity. A few facts, however, seem clear. Thus, the nitro derivatives of various groups were all active, while all the halogenated phenols tested gave reaction 2. The cyanines were not very effective. It appears that the sensitivity of the snails to this group was about intermediate between those of the highly susceptible filariid worms and the much more insensitive vertebrate tissues (Bueding, 1947, 1948).

The anthraquinones tested were less effective than the phenanthrenequinones and naphthoquinones. The hydroxynaphthoquinones, some of which are excellent inhibitors of the oxygen consumption of malaria parasites (Wendel, 1946) were less active than other members of the naphthoquinone series.

2-Methyl-1,4-naphthoquinone was a good inhibitor. The introduction of fatty acid side chains in position 6 had little influence on the activity up to and including the butyl derivative. The caproyl derivative showed a definite decrease in activity and the succinyl derivative had only a transitory effect. The thio derivatives with the thio group in position 3 were in general less effective than those having it in position 2, an exception being 2-methyl-3-thioethyl-6-butyryl-1,4-naphthoquinone.

Similar changes in activity due to changes in position of certain radicals on the naphthoquinone ring have been observed also in other cases; for example, by Geiger (1946) in respect to antibacterial activity, by Bueding (personal communication) in respect to antiglycolytic activity in schistosomes, and by Fieser and Richardson (1948) in respect to antimalarial activity. A comparison of the chemical constitution of the naphthoquinones inhibitory for various groups of organisms reveals numerous differences, indicating a specificity of action. This in turn may well serve as a support for the tentative view set forth above that we were dealing at least in part with enzyme inhibition in the case of our snails. It is to be expected that bacteria, malarial parasites, schistosomes, and snails do not all have exactly the same enzyme mechanisms.

SUMMARY

1. Seventy-two compounds, many of them potential molluscacides, have been tested as to their inhibitory effect on the oxygen consumption of *Australorbis glabratus*.

2. Four types of reaction could be distinguished: 1. No effect, 2. transitory inhibition, 3. slow inhibition, and 4. rapid and lasting inhibition.

3. Due to difficulties inherent in the animal material the mode of action of the compounds could not be ascertained with certainty. Some indications exist, however, that enzyme inhibition is involved in reaction types 3 and 4.

4. The most effective inhibitor found was α-nitrostilbene which inhibited the oxygen consumption by more than 99 percent.

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CUTEREBRA THOMOMURIS SP. NOV., A WARBLE FROM THE POCKET GOPHER, *THOMOMYS TALPOIDES* (RODENTIA: GEOMYIDAE)[1]

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The presence of warbles in pocket gophers, *Thomomys,* was first noted in 1857, when Leidy exhibited seven larval specimens at a meeting of the Academy of Natural Sciences of Philadephia. The demonstration, with a brief description and measurements of the larvae, was duly recorded in the Proceedings of the Society (1857). The warbles he exhibited had been collected by Dr. Hammond from under the skin of a pocket gopher found helpless along the roadside near "Bridger Pass" in the Rocky Mountains, July 1857. This locality is evidently in Carbon County, Wyoming, according to Bailey (1915), who recorded specimens of *Thomomys talpoides clusius* Coues collected at Bridger Pass, Carbon County, Wyoming, (18 miles southwest of Rawlins), by Dr. W. A. Hammond, July 28, 1857. The host of the warbles was recorded as "*Thomomys borealis* Richardson," which Bailey (1915) listed as a synonym of *Thomomys talpoides clusius* Coues.

In a reply to an inquiry (1945), Mr. J. A. G. Rehn, of the Philadelphia Academy of Sciences, stated that the specimens exhibited by Leidy are not in their collections. Leidy did not propose a name for these warbles other than assigning them to the genus *Oestrus,* nor was one proposed by Brauer (1863), who included the record and meager description under the genus *Cuterebra* in his Monographie der Oestridae. Cameron (1926) gave a rather complete description of *Cuterebra* larvae from a rat, *Rattus norvegicus,* collected in Saskatchewan and also included a record of collection and rearing of a single specimen of a *Cuterebra* from a "gopher" by Mr. Criddle in Canada. The host was probably *Thomomys.*

Six pocket gophers, *Thomomys talpoides,* some of which were infested with warbles, were brought to the Rocky Mountain Laboratory, September 4, 1935, by William LeClaire, from his ranch near Sula, Ravalli County, Montana. Three immature larvae were found in these carcasses. A few weeks later, Mr. LeClaire brought in three mature larvae from the same host species and locality, which would have been suitable for rearing but the larvae had already been preserved in alcohol.

A larger series of specimens has been collected by Prof. C. A. Tryon, of Montana State College, in the vicinity of Bridger Mountains, Gallatin County, Montana. Prof. Tryon collected and examined over 1000 pocket gophers. Of these, 15 were found infested with warbles; the larvae were in the subcutaneous tissues of the abdomen, back, knee, and thigh. Nine of the infested gophers were juveniles and 5 were adults, 2 were males and 12 were females. Age and sex were not recorded on one host. These larvae were measured by Prof. Tryon and measurements of 1 mm. to 23 mm. were recorded. Infested individuals were found from August 14 to October 27 and hosts were trapped in 1940, 1941, 1944, and 1945. Twenty-eight larvae were recovered from the 15 hosts; the largest number found in an individual animal was 6.

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[1] From the Rocky Mountain Laboratory, Hamilton, Montana, Microbiological Institute, National Institutes of Health.

The mature larva is here named and described on the assumption that it is a specific parasite of pocket gophers.

Cuterebra thomomuris sp. nov. (Diptera: Cuterebridae)

The holotype has been measured and photographed (figure 1, lateral view; figure 2, ventral view) and then dissected for more detailed study and illustration (figure 3). The chitinous parts were mounted on a series of micro-slides. Terminology for the parts described follows Patton and Evans (1929).

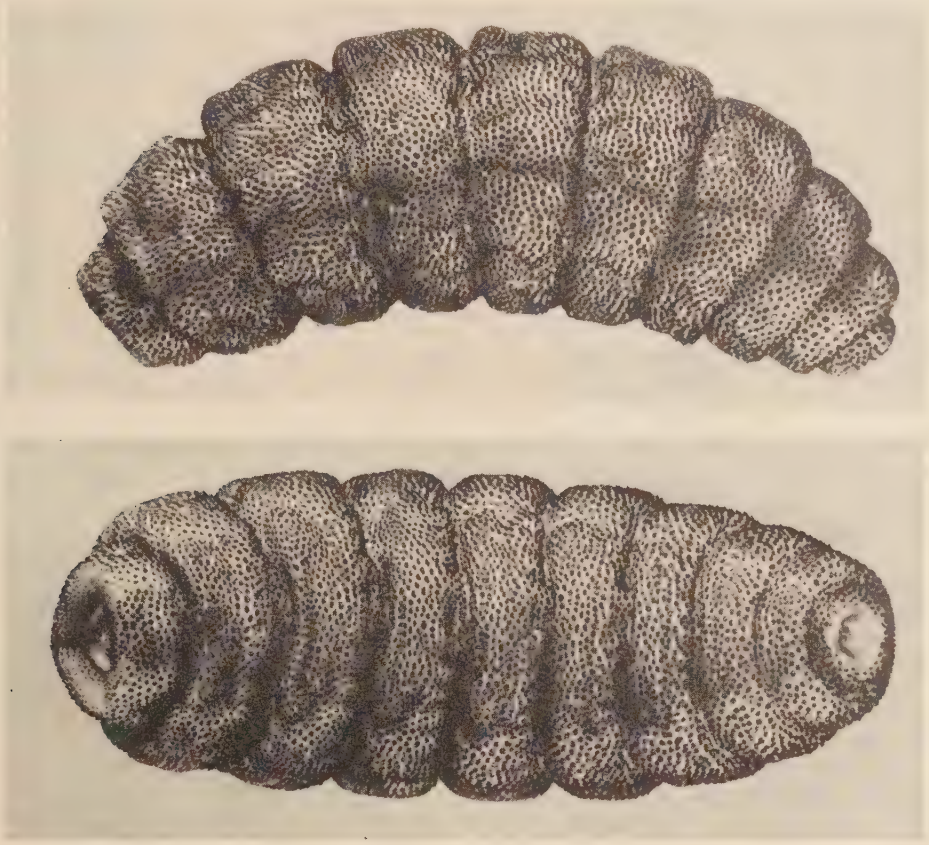
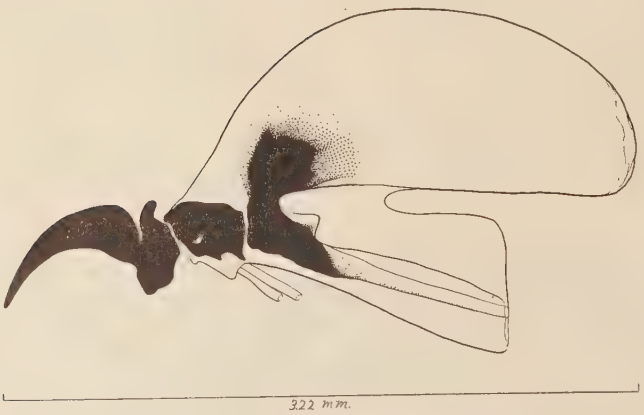


FIG. 1 lateral view and Figure 2, ventral view of *Cuterebra thomomuris* larva. Photographs by N. J. Kramis.

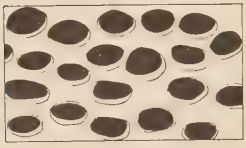
Length 23 mm., width 9 mm.; widest at the 8th segment. Thickness 8 mm., volume 1 cc., color chestnut brown. The sensory tubercles appear as slight eminences anterior to the oral hooks. Each tubercle bears two ocellus-like spots. Exteriorly, the oral hooks are shiny black and down curved. The dermis is ornamented with discrete dark platelets, most of which are circular or oval, but some bear spines and others have the posterior margin dentate. The anterior spiracles show as irregular darkened slits with a membraneous border in a fold between the second and third segments. They have no distinctive external structure but the aperature is definitely not sclerotized as in some other genera of bots e.g., *Cephenomyia*. The posterior spiracles are on sclerotized plates.

Dissection reveals a relatively large cephalopharyngeal skeleton 3.22 mm. long. The oral hooks are curved, without serrations, but with prominent dorsal and ventral prolongations at the base for attachment of muscles. The hypostomal sclerite is longer than high and has broad surfaces articulating anteriorly with the oral hooks and posteriorly with the pharyngeal sclerite. The



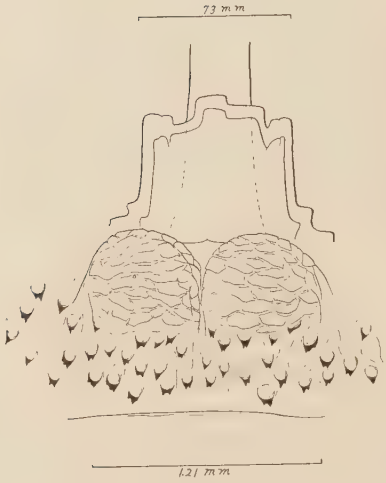
CEPHALO-PHARYNGEAL APPARATUS

TRACHEAL GLAND

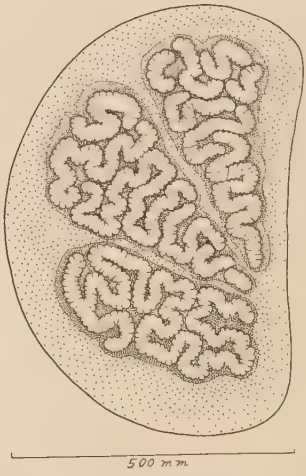


AREA OF DERMIS.

TYPES OF DERMAL PLATES



ANAL LOBES AND APODEME



POSTERIOR STIGMAL PLATE

FIG. 3. External and internal characters of *Cuterebra thomomuris* larva. Camera lucida drawings by Alma Dinehart

paired dorsal cornua of the apparatus are extended onto the anterior end of the hypostomal sclerite and posteriorly to enclose the dorsal pigmented portions of the basal sclerite. A posterior sinus divides a lower trough-shaped ventral cornu from the paired cornua. A duct extends along the groove of the ventral lobe.

The ends of the tracheae which terminate in the anterior spiracles are modified into dense glandular bodies measuring 1.064 mm. long. One of these is figured. In some species of *Cuterebra* and probably in this species, these glandular bodies are everted at pupation to form a pair of conspicuous feathery yellowish eminences on the outside of the pupa.

The posterior paired spiracles are somewhat recessed. Each spiracle measures .760 mm. by .500 mm., and the spiracles are separated by .045 mm. There is no button within the spiracle, this structure apparently being reduced to a very small sclerified plate between the spiracles. The wide margins of the peritreme are indistinct in contrast to those figured by Cameron (1926). Each spiracle bears 3 sinuous stigmal slits, each of which appears to be divided into several segments, and each slit is protected with numerous fine cross bars.

Just ventral to the posterior spiracle is a pair of low rugose anal lobes, and from this region a flat, almost rectangular, apodeme extends forward into the larva in a horizontal plane. This is figured.

Descriptions of all other North American *Cuterebra* have been based on the adult flies and adequate descriptions of larval stages of only 3 species have been made. The larvae of *C. buccata* (Fabricius) and *C. cuniculi* (Clark) were described by Knipling and Brody (1940). The larvae of *C. peromysci* Dalmat (1942) was described with the original species description of the adult.

The third stage larvae of *C. thomomuris* differs from these species in the following respects. It is smaller than *C. buccata* and *C. cuniculi,* measuring 19 to 24 mm. in length in contrast to 26 to 32 mm. and 35 to 42 mm., respectively. The anterior spiracle, posterior spiracular plate and cephalopharyngeal skeleton are also proportionately smaller. The typical dermal ornaments on *C. thomomuris* are slightly raised oval or dentate plates, while in *C. buccata* and *C. cuniculi* they are prominent sharp spines with one or occasionally two points. *C. thomomuris* has been found only in pocket gophers, rodents of the genus *Thomomys,* whereas *C. buccata* is a parasite of the cottontail rabbit, *Sylvilagus floridanus,* and *C. cuniculi* is a parasite of the marsh rabbit, *S. palustris,* both of the order LAGOMORPHA. Even in an area where these two rabbit parasites were present, cross infestation was not observed indicating a high degree of host specificity. *C. thomomuris* is comparable in size to *C. peromysci,* a parasite of the northern white-footed mouse, at Ames, Iowa. The latter measures 20–25 mm. in length. This species is described as having both numerous tuberculated spines and flattened, scale-like spines. The cephalopharyngeal skeleton is longer in *C. thomomuris* than in *C. peromysci,* 3.22 mm. in contrast to 2.5 mm.

There is a possibility that *C. thomomuris* is the larval stage of an adult that has already been named. Such synonymy will become evident when the species has been reared from known larvae. As so many adult *Cuterebra* have not been identified with their larval stages or with their mammalian hosts, it seems advisable that the study of the larvae, whose host relationships are known, be continued with the naming of species on larval characters when necessary even at the risk of creating synonyms with already named adults.

The type series consists of the holotype and two paratypes from *Thomomys talpoides,* Sula, Ravalli County, Montana, collected September 1935. The holotype is retained at the Rocky Mountain Laboratory. One of the paratype larvae, measuring 24 mm. length, 9 mm. width, 8 mm. thickness, is deposited in the United States

National Museum. The other paratype larva, measuring 19 mm. length, 8 mm. width, 7.5 mm. thickness, is in the collection of Professor C. A. Tryon, Montana State College. This specimen compares favorably with the largest specimen from *Thomomys* exhibited by Leidy, which was 19.1 mm. length, 11.6 mm. width, and 6.3 mm. thickness.

Six other mature larvae from *Thomomys talpoides,* Bridger Mountains, Gallatin County, Montana, collected by Professor Tryon, have been examined and are of this species.

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THE EFFECTS OF LARVAL POPULATION DENSITY ON SOME LABORATORY CHARACTERISTICS OF *ANOPHELES QUADRIMACULATUS* SAY[1]

LEVON A. TERZIAN AND NATHAN STAHLER

INTRODUCTION

The demonstration of the role of mosquitoes as disease vectors provided the impetus for extensive studies of these insects as laboratory animals. Notwithstanding, there has been little progress toward what might be termed the standardization of the mosquito for laboratory purposes. In particular, there may be considerable variation in the biting characteristics and behavior of *Anopheles quadrimaculatus,* even under well controlled laboratory conditions.

Since preliminary work indicated that larval development has specific effects on the adult mosquito, the present studies were undertaken in an attempt to define some of the intrinsic factors which affect the laboratory biology of *A. quadrimaculatus.* This report deals with the effects of two extremes of larval population densities on some morphological and physiological characteristics of this mosquito, and the effect of copulation on the avidity of the female for a blood meal.

MATERIALS AND METHODS

The strain of *A. quadrimaculatus,* used in these experiments, was obtained originally from the U. S. Bureau of Entomology and Plant Quarantine Laboratory at Beltsville, Maryland, in June 1945, and has been maintained in this laboratory since that time. Larval colonies were started from newly hatched first instar larvae, reared in white enameled photographic pans ($20 \times 17 \times 2\frac{1}{2}$ inches) containing five litres of tap water. The larvae hatching from the eggs produced during a single night in a stock breeding cage, reared simultaneously in three or more pans under the same conditions, constituted a larval colony. Each pan of larvae received 200 mgm. of Difco Brain Heart Infusion and 20 mgm. of finely powdered brewer's yeast on the first day. Beginning with the first day, and thereafter, the larvae were maintained on finely powdered (100 mesh) Purina Laboratory Chow, added more frequently and in increasingly larger amounts, 100 mgm. to 250 mgm. per dose per pan, as the larval colonies grew older (Heal and Pergin, 1945).

Colonies designated as underpopulated contained an average of 150 larvae per pan, with approximately 2.3 square inches of surface available per larva, while the overpopulated ones contained an average of 1500 larvae per pan, with about 0.2 square inch of surface per larva. In order to have approximately the same amount of food available per larva in both underpopulated and overpopulated colonies, the latter were refed as frequently as the surface was swept clean, while the larvae in the underpopulated pans were fed three or four times during the working day. In either type of colony there was no significant mortality during the period of larval development, even though there was vigorous competition for space and food in the overpopulated pans. In underpopulated colonies pupation usually began on

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the eighth day and was completed by the tenth day, while in overpopulated colonies pupation would not ordinarily begin until the tenth day and would then continue until the twentieth or twenty-first day before pupation was fully completed.

Feeding experiments were conducted on the fifth day after emergence was completed, in 18-mesh wire screened cages of the sleeve type ($12 \times 12 \times 12$ inches) with large, water-soaked cotton pads placed on top. Except when otherwise indicated, moist raisins were always kept in the cage until the host chick was inserted. White leghorn chicks, weighing 75 to 150 grams, were tied securely to boards, plucked free of feathers on the exposed side, and placed in the cages for exactly thirty minutes, at 11 a.m. on the day of the feeding. Exactly sixty females, with the requisite number of males, were used in all biting experiments except in those on the effect of the male/female ratio on biting, in which the numbers of females varied between 55 and 130.

Larvae were reared, and all the experimental procedures were carried out, at a constant temperature (80° F., dry bulb), and a constant humidity (70° F., wet bulb). The light source was a Mazda, 40 watt, white, fluorescent lamp about 10 feet from the cage, and light intensity at the feeding site was measured by means of a Weston microammeter and a Weston photronic cell.

RESULTS

In early experiments designed to determine the effects of larval populations on the biting characteristics of adult *Anopheles quadrimaculatus,* pupae were picked at random, and groups from each day's harvest were then set up in paired cages for biting trials. Since the ratio of the sexes changes gradually from day to day in overpopulated larval colonies as successive crops of larvae pupate, such random selection resulted in groups of mosquitoes with considerable variation in the proportions of males and females. The erratic biting behavior of these groups made it appear that the relative distribution of males and females in a given group might be a factor in determining the females' propensity to take a blood meal. This was borne out by a number of experiments in which the numbers of male per female within a given group were varied over a wide range of proportions. In these experiments, the pupae from underpopulated colonies were differentiated sexually under the dissecting microscope before being set up in cages in varying proportions of sexes. In order to keep at a minimum the effects of as many other factors as possible, the pupae were allowed to emerge in total darkness, and the adults were maintained and offered a blood meal under the same conditions.

It is evident from these experiments that the relative proportion of male to female is an important factor in determining the blood avidity of the female (fig. 1). The lowest biting rates occur in those groups with the lowest percentage of males, and coincident with increasing percentages of males there is an increase in the biting rate, while the highest rates occur among those groups with the highest proportions of males. There is a reliable positive correlation between the biting rate of the females and the percentage of males present within the group ($r = .84$). In these biting trials, virgin females which had had no contact with males, never took a blood meal. Since, in other experiments, virgin females have been observed to feed, although poorly, the complete lack of response to a blood meal in these cases may have been due to the lack of more favorable biting conditions.

There is evidence to indicate that copulation rather than the presence of males alone is the significant factor in stimulating females to take a blood meal. This is shown by experiments in which, one-half hour before feeding, various numbers of males were withdrawn from one of a pair of cages containing fifty females, by others in which fifty males were added to one of a pair of cages containing fifty virgin females, and by some in which various numbers of males were added to one of a pair of cages containing equal numbers of both sexes. In no case did the change in the proportions of male and female under these conditions produce any significant difference in the biting rate between the paired cages. As a consequence of the importance of mating, and of the male/female ratio in determining biting rates, the

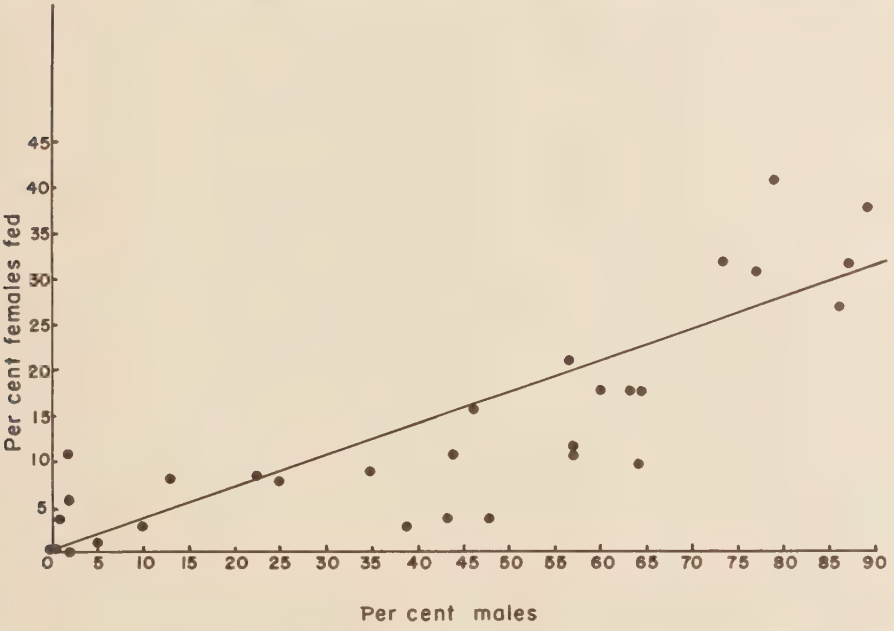

Figure 1.- The effect of the male-female ratio on the biting rate of A. quadrimaculatus.

proportions of males to females were always controlled in the experiments designed to evaluate the biting characteristics of adults from underpopulated and overpopulated larval colonies of *A. quadrimaculatus*.

The studies on population effects indicate that the stresses imposed on the individuals in a larval colony, by the population density of that colony produce significant differences in the length of the developmental period between larvae from underpopulated and larvae from overpopulated colonies, and in turn, significant differences in the pupae and the adults from such colonies. This is reflected, first, in the biting characteristics of adults derived from underpopulated and overpopulated larval colonies. A comparison of the biting rates of adults from both types of colonies indicates that even though there is variation in both groups, nevertheless, there is evidence to indicate that under certain conditions the biting rate of adults from underpopulated colonies is significantly greater ($P = .02$) than the biting rate of adults from the overpopulated colonies (table 1). It should be

noted, however, that with certain changes in the conditions of an experiment this difference may be obscured. It is more important to note that the variation in the biting rate within a single overpopulated colony is considerably more than that within the underpopulated colonies. The coefficient of relative variability for underpopulated colonies is 0.33 and for overpopulated colonies it is 0.88.

TABLE 1.—*Effect of larval population density and larval age on feeding rates of* Anopheles quadrimaculatus

Larval age	Per cent females feeding				
	Underpopulated			Overpopulated	
	I	II	III	I	II
8	24				
9	20	31	40		
10	16	18	26		
11				13	42
12				9	
13					10
14				8	2
15				2	6
16				30	21
17				11	14
18				3	3
19				16	
Average	25.0 ± 8.3			12.7 ± 11.2	

It is of interest to note that a curve indicating the change in biting rate in overpopulated colonies resembles the curve of the numbers of individuals pupating from day to day from that colony (fig. 2). Although the reasons for this are not entirely clear, nevertheless, the evidence from these, and from numerous other experiments, indicates that with relatively poor biting conditions, namely, a ratio of one male to one female, feeding in total darkness (fig. 2a), or under a light intensity of 3.1 foot candles (fig. 2b), there is a consistent relation between the biting rate and the numbers pupating each day in a colony. For figure 2a, the correlation coefficient between the percent biting and the percent pupating is .97 and for figure 2b it is .91. The biting rate is lower for those individuals derived when the least numbers are pupating, and highest for those individuals pupating on the days of maximum pupal production. Under conditions better suited to induce biting, namely, a more favorable light intensity of 1.5 foot candles (fig. 3a), or a two to one ratio of males to females with raisins removed 24 hours prior to the blood meal (fig. 3b), the biting responses of an overpopulated colony become more erratic. Although the biting rates reach higher values, there are greater variations from day to day and the peaks of biting are no longer coincident with the days of maximum pupation. The correlation coefficient for figure 3a is .72 and for figure 3b it is .03. All the correlations are based on days common to all the figures. Although the biting rates of adults from underpopulated colonies are also considerably increased, when offered a blood meal under more favorable conditions, the variations are not as great among these groups. Thus, it would appear that population density during the larval development of *A. quadrimaculatus* is an important factor in determining the biting characteristics of the adult.

In addition to its effects on the biting propensities of the adult, population density during larval development has a significant effect upon the physical characteristics of the pupae, manifested by the considerable differences in size between the

individuals from undercrowded and overcrowded colonies. Relative size was determined by measuring the paddle from the base of the buttress to the apical seta. The relation of length of the paddle to the volume of the whole pupa has not yet been established, but this organ appears to be the only adequate landmark which permits of simple and accurate measurements. On the basis of such measurements, it

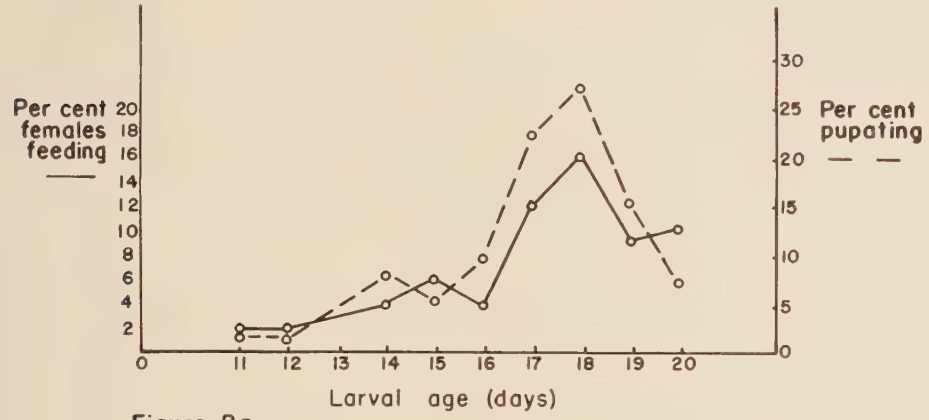

Figure 2a.

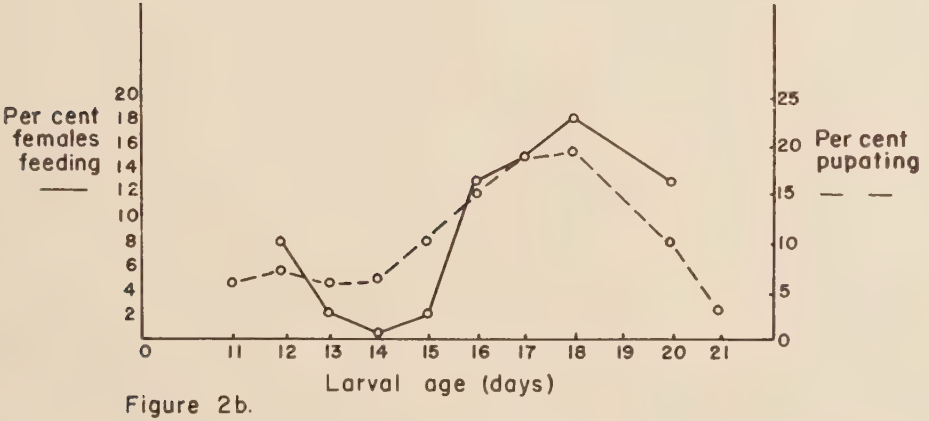

Figure 2b.

Figure 2.- The change in biting rate of successive crops of females compared to the numbers pupating from day to day in overpopulated colonies of A. quadrimaculatus.

is evident from the data that pupae from underpopulated colonies are significantly larger ($t = 3.91$ with $P =$ approximately .0005) than the pupae from overpopulated colonies (table 2). Although the individuals among the last to pupate in an overpopulated colony are larger than those completing development earlier, nevertheless, these older pupae are still significantly smaller than those from the underpopulated colonies. However, it should be noted from the measurements on pupae from

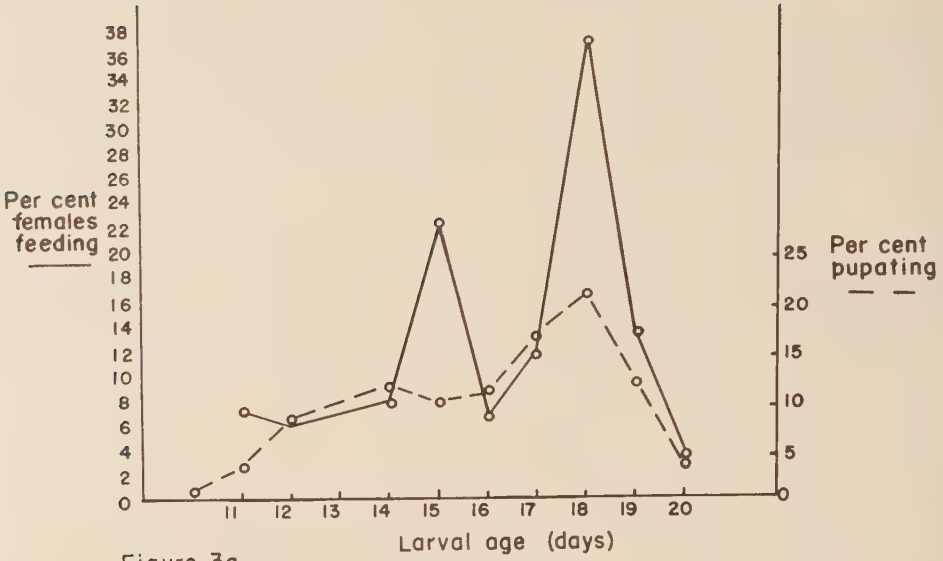

Figure 3a.

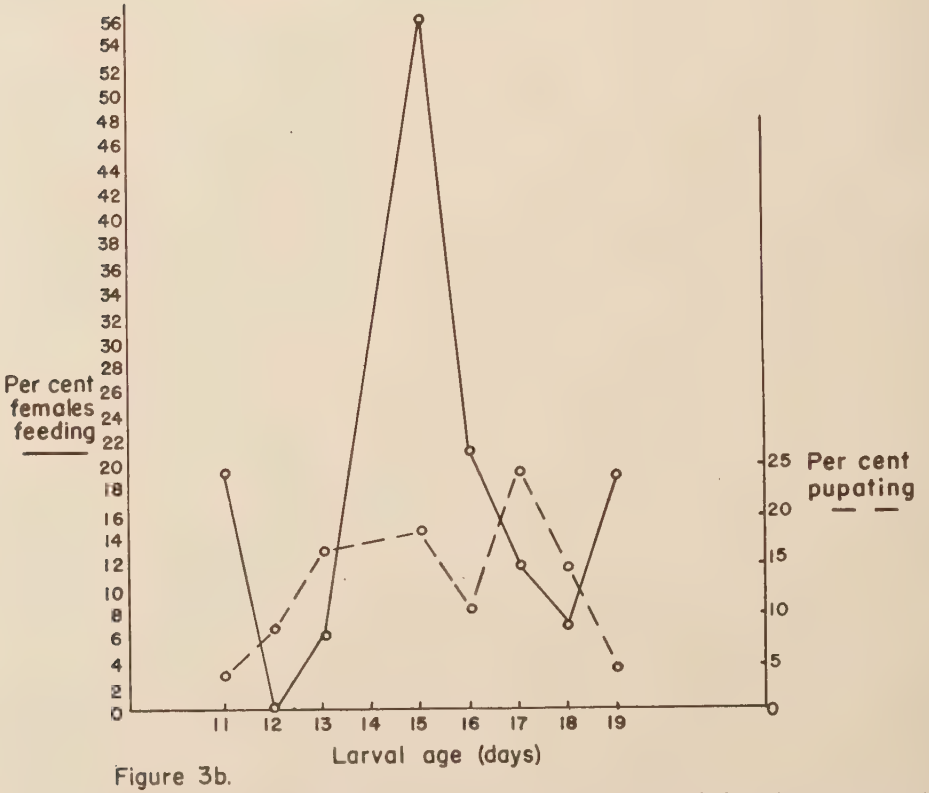

Figure 3b.

Figure 3.- The biting rate of successive crops of females compared to the numbers pupating from day to day in overpopulated colonies offered a blood meal under more favorable conditions.

overcrowded colonies that paddle size alone is not a sufficient criterion for demonstrating small differences in pupal size. Again, as with the variation in the biting characteristics of the adults, it is evident by inspection (table 2) that there is more variation in size among the pupae from overpopulated colonies than there is in those from underpopulated ones. In either case, male pupae are consistently smaller than the females.

TABLE 2.—*The effect of larval population densities and larval age on the weight and size of* Anopheles quadrimaculatus *pupae*

Larval age	Weight (mgm.) 20 pupae		Size (length of paddle, mm.)	
	Under-populated	Over-populated	Under-populated	Over-populated
8	1.32		0.84 ± .02	
9	1.30		0.86 ± .02	
10	—		0.84 ± .03	
11		0.39		0.72 ± .03
12		0.38		0.70 ± .03
13		—		
14		0.32		0.68 ± .04
15		0.40		0.70 ± .02
16		0.52		0.71 ± .03
17		0.59		0.72 ± .03
18		0.61		0.75 ± .04
19		—		
20		0.64		0.76 ± .04
Average	1.31	0.48	0.85	0.72

As would be expected, in view of the difference in size, pupae from underpopulated colonies are heavier than those from overpopulated ones (table 2). Lots of 20 female pupae were picked daily from an overcrowded colony, dried for 24 hours at 40° C., weighed on an analytical balance, and compared with the weights of two lots from an undercrowded colony. Since, in general, pupae from underpopulated colonies were approximately three times heavier than the pupae from overpopulated pans, the obvious differences between the two groups are more evident from the measurement of weight than they are from the measurement of the length of the paddle. Again, the individuals in overpopulated colonies which are the last to pupate are significantly heavier than those pupating earlier.

In addition to the obvious influence on size and weight, it was found that population density had a significant effect on pupal viability, in that as the period of larval development lengthened in overpopulated colonies, more and more individuals in emergence dishes filled with tap water, died during the pupal stage, or failed to complete emergence from the pupal case (table 3). These data indicate that pupal mortality is greater, and more directly related to the length of the larval period, in the males than in the females, but with both the mortality increases significantly with larval age ($r = 0.93$). Further, the mortality which occurs among the individuals with longer larval periods is greater than the mortality in larvae with shorter larval periods and those derived from underpopulated colonies. It does not appear probable that the excessive mortality which occurs in the groups which are among the last to pupate could be due to so-called autointoxication (Roubaud, 1923) since it has been possible, in this laboratory, to rear more than five generations of overcrowded larvae in the same medium without undue mortality.

It is of interest to note, too, that these data confirm the generally recognized fact that the males pupate earlier than the females. In overpopulated colonies, the

TABLE 3.—*A comparison of the effects of larval age and population density on the viability of* Anopheles quadrimaculatus *pupae*

Larval age	Number samples	Underpopulated						Overpopulated					
		Total specimens			Per cent dead			Total specimens			Per cent dead		
		Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
8	2	150	64	214	1.3	3.1	1.9						
9	5	558	404	962	1.6	2.5	2.0						
10	7.1*	698	926	1624	5.4	1.7	3.3	298	74	372	1.3	0.0	1.1
11	6							762	254	1016	3.3	5.5	3.8
12	7							961	342	1303	4.0	3.5	3.6
13	5							625	366	991	16.0	8.5	13.2
14	10							1024	895	1919	12.8	7.4	10.3
15	11							888	1086	1974	18.5	8.6	13.0
16	12							551	1745	2296	25.4	5.4	9.5
17	12							793	1474	2267	22.4	7.7	12.2
18	12							621	1416	2037	35.6	13.4	19.7
19	6							161	784	945	41.0	10.6	15.8
20	9							175	1110	1285	45.7	14.8	19.0
Total		1406	1394	2800	4.8	3.3	4.0	6859	9546	16405	17.4	9.3	12.7

* Sample for overpopulated colony

average length of the larval period was 14.4 days for the males, and 16.5 days for the females.

Finally, there is evidence to show that the larval history has an effect on adult longevity. It is difficult to demonstrate any clear differences in the total life span between adults from underpopulated and from overpopulated colonies maintained on raisins and water, or given sugar for the first two post-emergence days and then maintained on water alone for the balance of the time. When maintained on water alone, however, the adults from underpopulated colonies survive approximately twice as long as the adults from overpopulated colonies (table 4).

TABLE 4.—*The influence of larval age and population density on longevity of virgin adult females of* Anopheles quadrimaculatus

Larval period	ADULT DIET									
	Water only				4 per cent sugar for 2 days followed by water					
	Underpopulated		Overpopulated		Underpopulated			Overpopulated		
	No.	Average longevity	No.	Average longevity	No.	Average longevity	Per cent dead in 5 days	No.	Average longevity	Per cent dead in 5 days
8	44	4.8			21	16.2	0			
9	24	3.3			21	19.8	0			
10	25	3.2			25	17.1	4			
11								26	20.0	0
12			19	1.1				25	21.4	0
13										
14			16	1.3				27	20.1	0
15			26	1.9				19	19.5	5
16			24	1.8				21	20.6	10
17			18	1.8				28	16.5	14
18			25	1.8				30	15.9	23
19								25	10.3	44
20			32	1.7				21	12.1	43
Average		3.9		1.7		17.7			17.3	

There are, in addition, differences in the survival time of adults from overpopulated colonies. These differences are not evident if the adults are maintained on raisins and water, or water alone, but given sugar the first two post-emergence days and then maintained on water, there are significant differences, related to the period of larval development, in the survival time of adults from overpopulated colonies

(table 4). Thus, the longer the period of larval development, the shorter the average survival time ($r = -0.85$). This relation is even more strikingly evidenced by the data indicating the degree of mortality during the first five days of adult existence. Whereas a large percentage of the adults with larval periods of 18, 19 and 20 days were dead at the end of five days, none had died in the groups with larval periods of 11, 12 and 14 days. In general, therefore, it appears that the longer the period required by larvae to complete their development under overcrowded conditions, the less the probability that they will be able to survive beyond the first few days of adulthood.

COMMENT

It is evident from the data presented that larvae reared in overcrowded colonies produce pupae and adults which are characteristically different, physically, from the pupae and adults derived from undercrowded colonies in which conditions are nearly optimal for laboratory growth. These differences, due essentially to population stresses and their consequent effect on the period of larval development are further demonstrated by the erratic biting behavior of adults derived from overpopulated colonies as compared to the biting behavior of adults derived from underpopulated colonies. If the biting propensities of the females, under standardized conditions, be considered as an adequate criterion of their adult physiological characteristics, then the erratic biting pattern of individuals from overpopulated colonies is an indication of the extent of physiological variation among the adults derived from such larval colonies. Thus it would appear from these studies that the greater the stresses imposed on the individuals during larval development, the greater will be the physical and physiological variations among the resulting adults.

CONCLUSIONS

1. It has been shown that the pupae and adults of *A. quadrimaculatus* derived from larvae reared in colonies with low population densities differed characteristically from the pupae and adults derived from colonies with high population densities. The pupae from underpopulated colonies were larger and heavier, and had significantly lower mortality rates than the pupae from overpopulated colonies. The adults had a higher biting rate and showed more uniform biting propensities than the adults from overcrowded colonies.

2. There was significantly greater variation in the size and viability of the pupae, and in the viability and biting rates of the adults from overpopulated colonies as compared to the pupae and adults from underpopulated colonies. In addition, under certain conditions, the curve indicating the biting rate of successive crops of females from overpopulated colonies paralleled the curve indicating the rate of pupation in such colonies.

3. It has been shown that the male/female ratio is related to the biting rate, and there is evidence to indicate that copulation, rather than the mere presence of males, is the significant factor in stimulating females to take a blood meal.

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DESCRIPTION OF THE MALE OF *COSMOLAELAPS GURABENSIS* FOX (ACARINA, LAELAPTIDAE)[1]

D. C. Thurman, Jr.[2] J. A. Mulrennan[3] and Nina Branch[4]

The female of *Cosmolaelaps gurabensis* Fox, 1946, was described (Fox, 1946) from collections of ectoparasites of rats and mice taken at Camp O'Reilly, Gurabo, Puerto Rico, in 1945. During the course of surveys and evaluation studies of the control of rat ectoparasites with DDT, a total of sixty specimens of *C. gurabensis* was taken in Florida. This material included two males. The descriptions of the male of this mite is given herein.

FAMILY LAELAPTIDAE

Cosmolaelaps gurabensis Fox

Male: Body length approximately 0.51 mm; body width, 0.33 mm.

Dorsum.—Dorsal shield reticulated, entire, covers the dorsal surface of the body, bears 30 to 35 pairs of curved clavate ribbed setae.

Venter.—Presternal plates (Jugularia) distinct, wing-shaped, apparently united on the mid-ventral line. Holoventral plate heavily sclerotized, distinct, with a network of fine lines, ten pairs of long setae (in addition to the three anal setae) arranged as shown in figure 1; two pairs of pores present. Anal pore, oval, slightly smaller than genital pore. Six to eight pairs of setae set in the soft integument of the abdomen. Peritremes extend from the level of the mid-points of the first coxae to the level of the posterior margins of the fourth coxae; stigmal pores on an imaginary line drawn across fourth coxae. Basal segment of tritosternum reaches the level of the base of the gnathosoma; second segment two branched to base, twice the length of the basal segment, with long, prominent widely spaced pinnae, reaches the apices of the first palpal segments.

Gnathosoma.—Hypostome bearing five transverse rows of teeth, 6 to 12 teeth in each row. Labium membranous, six branched inner branches long, thin, with many pinnae, second branches long, with many pinnae, outer branches short. Lingula sword-shaped, extending normally beyond the apices of corniculi, tip flexed. Chelicerae with heavily sclerotized, pigmented chelae about twice as long as the width of the basal segment. Fixed digit with small seta (pilus dentilis) located just beyond the middle, also with a blunt tooth-like projection posterior to seta. Movable chela with a row of setae forming a brush at its base. Apparently a single spine-like seta at the base of the fixed digit when in lateral view. Epistome membranous, pointed, apical margins serrate (similar to that of female).

Legs.—Chaetotaxy normal. In order of length, I, IV, II, III; in breadth, II much broader than the others, I and III slender, IV slightly stouter than I and III. Coxae I has an epidermal projection interiorly near its base.

Type material.—Allotype from *Rattus norvegicus,* Pensacola, Florida, April 2, 1948 (A. E. Carnley). Paratype from the nest of *Rattus rattus alexandrinus* from Rock Harbor, Key Largo, Monroe County, Florida, December 2, 1947, (James S. Haeger). Both specimens are deposited in the collection of the U. S. National Museum, Washington, D. C.

Fifty-eight females of *C. gurabensis* have been collected from rats or rat nests in Florida from the following localities: Pensacola, Rock Harbor, Jacksonville, Miami and Tampa.

Of the above collections, ten females, five from Rock Harbor, two from Jacksonville and one each from Pensacola, Miami, and Tampa were examined by Dr. Irving

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Fox and found to be con-specific with *C. gurabensis* when compared with his type specimens. Five of these specimens were retained by Dr. Fox for the collection of the School of Tropical Medicine, San Juan, Puerto Rico, while the other five have been sent to the U. S. National Museum, Washington, D. C.

DISCUSSION

The male of *C. gurabensis* is smaller than the female. Some variations in the rows of teeth on the hypostome were observed. There appeared to be six transverse rows on the paratype male while the females studied had five, six and seven rows. The movable chela of the chelicera seemed to be composed of two fused arms, as apparent in the illlustration. However, this has not been observed in examinations of other mites. The epistome is difficult to see unless removed from the rest of the gnathosoma.

Cosmolaelaps was first established by Berlese as a subgenus of *Laelaps* in 1903 (1904) and is closely related to *Hypoaspis*. It differs from others in the HYPOASPIDINAE Vitzthum (1940–1943) in the unusual forms of the dorsal body hairs. Berlese described these hairs from the various species as simply dilated, club-shaped and ribbed, cultriform or in the shape of rough tubercles. *C. gurabensis* has clavate hairs, with ribs, the most distinct of these hairs being located dorsally on the posterio-lateral margins (figured by Fox). The dorsal hairs of *Cosmolaelaps cuneifer* (Michael, 1891) were referred to as "clavato-costulati" by Berlese despite the fact that Michael figured and described them as wedge-shaped. These hairs (as figured and described) are apparently quite different from the hairs found on *C. gurabensis.*

The literature was found to contain the following species, the validity of which remains for future acarologists to determine:

Genus *Cosmolaelaps* Berl. 1904 (1903) Zool. Anz. Bd. 27: 13.

Cosmolaelaps claviger (Berl.) *Laelaps claviger* Ac. Myr. et. Scorp. It. fac. IV. 2 typus.

Cosmolaelaps cuneifer (Mich. 1891) *Laelaps cuneifer* Proc. Zool. Soc. Lond. p. 647.

Cosmolaelaps scalpriger (Berl. 1902) *Laelaps scalpriger* Zool. Anz. 25: 699 (fem.)
C. scalpriger Berl. 1902 (1903) Zool. Anz. 27: 19 (Male).

Cosmolaelaps vacua (Michael 1891) *Laelaps vacua* Proc. Zool. Soc. Lond. p. 646.

Cosmolaelaps ornatus Berl. 1904 (1903) Zool. Anz. 27: 19.

Cosmolaclaps simplex Berl. 1921 Redia 14: 157.

Cosmolaelaps vacuus (Mich. 1891) var. *hastiger* Berl. 1921 Redia 14: 157.

Cosmolaelaps simplex Berl. 1903 var. *digrediens* Berl. 1923 Redia 15: 253.

Cosmolaelaps gurabensis Fox 1946 J. Parasit. 32: 449.

With regard to the mouthparts, it is interesting to note that in the descriptions and drawings of the chelicerae of the males of *C. vacua* and *C. cuneifer,* Michael described the movable arm as having a long, slender, round, and undulated accessory piece on its outer side, which projects considerably beyond the rest of the chela. No similar accessory piece was noted on the specimens of *C. gurabensis* examined.

Acknowledgments are due to Dr. R. W. Strandtman for his suggestions with regard to this description and particularly with that part dealing with the gnathosoma, to Dr. Harry D. Pratt for his careful criticism of the manuscript, and to Dr. E. W. Baker for his assistance in our studies of the ACARINA.

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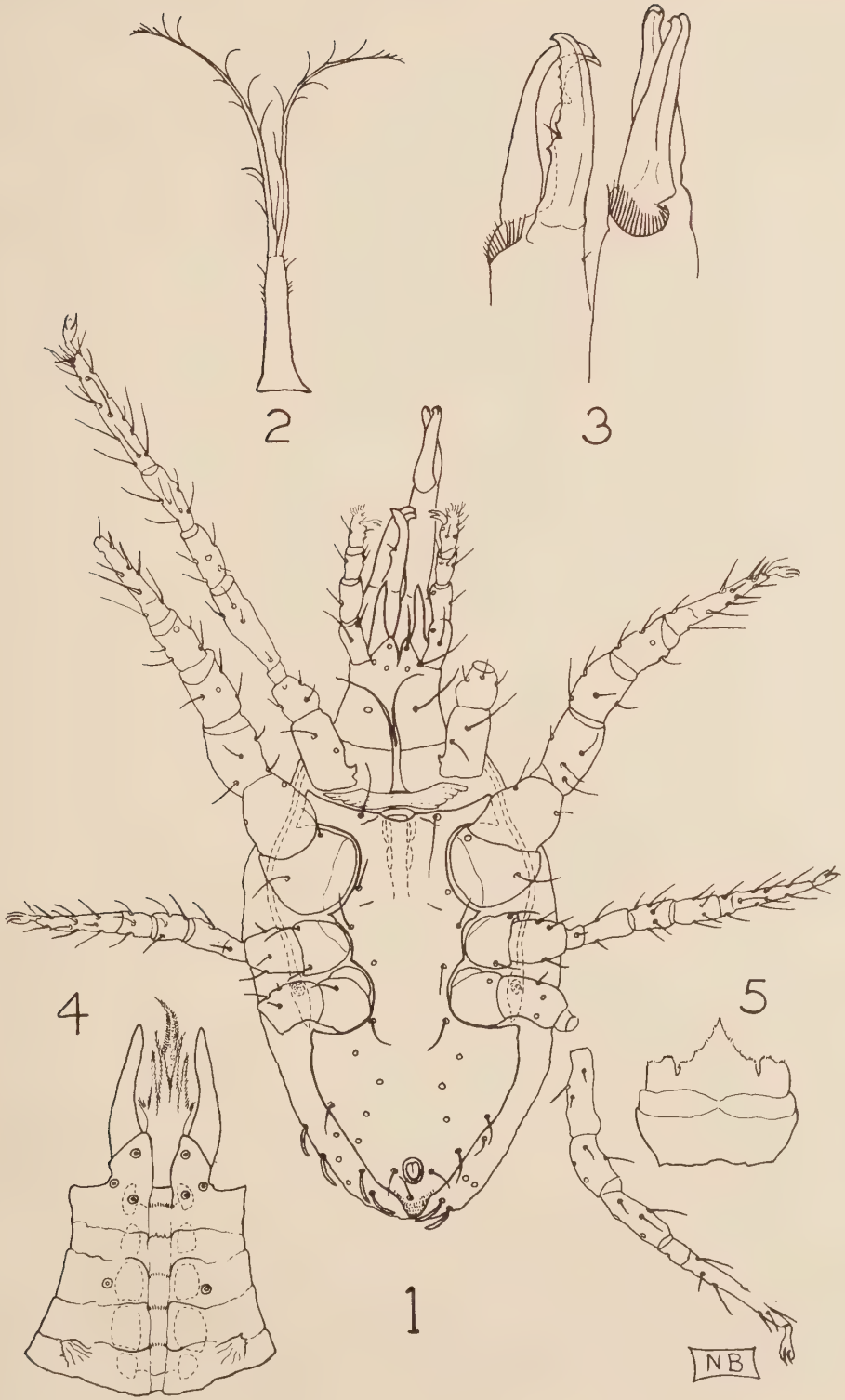
Plate I.

Male of *Cosmolaelaps gurabensis* Fox (Figs. 1–3 from allotype, Figs. 4 and 5 from paratype.)

Fig. 1. Venter.
Fig. 2. Tritosternum.
Fig. 3. Chelicerae.
Fig. 4. Ventral view of gnathosoma.
Fig. 5. Epistome.

PLATE I



THE ACANTHOCEPHALAN GENUS *NEOECHINORHYNCHUS* IN THE CATOSTOMID FISHES OF NORTH AMERICA, WITH DESCRIPTIONS OF TWO NEW SPECIES

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In 1931 the writer described *Neoechinorhynchus australis* as a new species from an undetermined species of the genus *Ictiobus*. At that time relatively little attention had been directed to the striking diversification that has marked speciation in the NEOECHINORHYNCHIDAE along with the development of a distinctive North American fresh water fish fauna. More recently, correlation between speciation of fish hosts on this continent and parallel development of their acanthocephalan parasites has been emphasized in several papers (Van Cleave 1945, 1947, and Van Cleave and Manter 1948). New evidences are constantly being brought to light on this problem through discovery of entirely new sources of material and likewise through reinterpretation of earlier materials. In the present study, two new species of *Neoechinorhynchus* are recognized from members of the sucker family (Catostomidae). One of these, *N. distractus,* is separated from *N. australis,* a confused composite species as conceived in the literature, and *N. strigosus,* an entirely new concept based upon 13 females from the smallmouth buffalo (*Ictiobus bubalus*) of Reelfoot Lake, Tennessee, collected by William B. Robertson and a long series of both males and females taken in Wisconsin by Ralph V. Bangham. These included 46 females and 10 males from the white sucker (*Catostomus commersonnii*) of Lake Chetac, Sawyer County, Wisconsin and a series of more than 90 individuals from the northern redhorse (*Moxostoma aureolum*) from the Mississippi River at Fountain City, Buffalo County, Wisconsin. In other individuals of the northern redhorse taken from the Mississippi River at Stoddard, Vernon County, Wisconsin, a few specimens of *N. strigosus* occurred along with an undetermined species of NEOECHINORHYNCHIDAE having an elongated proboscis with a large number of hooks. Unfortunately the proboscides in this latter material were all introverted or retracted within the body so that details for recognition of the species and genus are unavailable at this time.

For a very long time it has been recognized that the two lemnisci in individuals of all members of the family NEOECHINORHYNCHIDAE are morphologically distinguishable from each other. This usually finds expression as a slight difference in size, correlated with the fact that the slightly smaller lemniscus contains a single giant nucleus while the larger has two. This normal distinction has been commonly regarded as explainable on the basis of the nucleus-cytoplasm ratio in the two organs (Van Cleave, 1914) although volumetric calculations have never been presented in support of this assumption.

In fully mature individuals of most species of *Neoechinorhynchus,* the differential in size of the two lemnisci is slight enough that relative extent of contraction or of looping and twisting may obscure the basic difference in length. Against this basic pattern of slight, though significant, distinction between the two lemnisci of the same individual, the conspicuous diversity of the lemnisci in *N. australis,* and some

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other species, stands out prominently because one lemniscus is regularly several times the length of the other and the volumes of the two are radically different (see Figs. 1–4).

Many of the early writers recorded what seem to be erroneous and inconsistent observations on the sizes of the lemnisci and the numbers of their nuclei in *Neoechinorhynchus*. Some of these mistaken records are apparently due to inaccuracy of observation while others may have resulted from the will to regard the lemnisci as identical members of a series of laterally paired structures. So far as the present writer is aware, there has been only one critical study on variability in the lemnisci of this genus. Lynch (1936: 40) studied three species of the genus and found that 10 of the 40 individuals examined critically revealed deviations from the customary numbers of two nuclei in one lemniscus and one in the other. If this statement is taken alone, without analysis, it would seem to throw considerable doubt upon the validity of an assumption of fixed pattern of nuclear complements in the NEOECHINORHYNCHIDAE.

In seven of the ten individuals of *Neoechinorhynchus* in which Lynch found aberrant arrangement of the nuclei, the increase or decrease in their number within the lemnisci was associated with a corresponding alteration in number of the subcuticular nuclei. These seven instances seem to represent misallocations in the final shuffling and rearrangement of the embryonic giant nuclei of the hypodermal rudiments during organogenesis and do not constitute fundamental deviations from the predetermined, over-all, plan of organization (Van Cleave, 1914) within the family.

The most significant conclusion to be drawn from Lynch's table concerns the apparent tendency for the two lemnisci to always show some degree of basic size dimorphism which is commonly assumed to be directly associated with a difference in nuclear numbers. This tendency has become so firmly fixed that in instances of rearrangement of the nuclei of the lemnisci and subcuticula, the two lemnisci retain their distinctive size dimorphism even in those abnormal individuals which possess identical nuclear complements. Lynch was able to distinguish a large and a small lemniscus in individuals which had either one or two nuclei in both organs. From this observation it would seem that the predetermined basis for size differential of the two lemnisci might have been originally established as an expression of nucleus-cytoplasm relation, but once fixed the differential persists even when the inequality in nuclear numbers is no longer present in the individual organs.

The first evidence of an extreme diversification of the two lemnisci in the same individuals was presented (Van Cleave, 1931) when *N. australis* was described. At that time *N. australis* was the only species of the entire phylum for which extreme disparity in size of the two lemnisci in the same individual had been demonstrated. Later, Lynch (1936) described two speices, *N. venustus* and *N. cristatus*, with varying degrees of dissimilarity between the lemnisci. Several clearly distinct morphological characters are available for differentiating between *N. australis*, *N. venustus*, and *N. cristatus*. This emphasizes the fact that inequality of the two lemnisci in the same individual is not a simple specific character but constitutes an additional morphological feature available for expressing specific differences in some NEOECHINORHYNCHIDAE. This interpretation was not available in 1931 when *N. australis* was first recognized. At that time the feature seemed so unusual and so consistently different from anything that had been observed previously that diversi-

fication of the lemnisci was regarded as a distinctive specific character. Similar erroneous evaluations of newly discovered features are encountered frequently in the literature on acanthocephalan taxonomy.

Blinded by the misinterpretation of the significance of this feature, the writer failed to evaluate other differences which were present in the original material on which *N. australis* was founded. As a result, two distinct species were confused under a single name. More recent study of the material and reevaluation of the essential morphological features have made it clear that *N. australis,* as originally described, is a composite of two distinct species having in common the feature of diverse lemnisci but differing from each other in several other essential morphological details. In the present article the australis-complex is resolved, *N. australis* is redescribed and some of the original specimens are segregated as type and paratypes of a distinctively new species under the name *Neoechinorhynchus distractus.*

In the species of *Neoechinorhynchus* which have established a conspicuous size difference for the lemnisci, this character seems to have lost its original close integration between volume of the cytoplasm of the lemnisci and volume or surface of their nuclei. The derived condition might represent an orthogenetic accentuation of the relatively slight differences inherent within the genus without involving further direct correlation of size and function of nuclei and cytoplasm.

There seem to be no other quantitative expressions of taxonomic value accompanying the slight, the moderate, and the extreme degrees of diversification of the lemnisci in the various species of *Neoechinorhynchus.* Consequently, dimorphism in the lemnisci cannot be considered as of generic significance since it occurs in varying degrees as a fluctuating variable in several species of *Neoechinorhynchus* lacking the supporting evidence of other distinctive features to justify recognition of any natural grouping of the species other than as parallel members within the relatively large genus *Neoechinorhynchus.*

Resolution of the australis-complex

In the original study of *N. australis,* size and shape of the body, disposition of the male reproductive organs with respect to total extent within the body cavity and their spatial relations to the lemnisci, size and shape of the proboscis and size of its hooks are variables whose importance was ignored because of the overemphasis upon the single feature of relative length and size of the two lemnisci. It was recorded, as an incidental observation, that in some individuals of the original material the length of the larger of the two lemnisci was more exaggerated than in other specimens but this was interpreted as an instance of individual variability or as possibly due to extent of contraction as expressed in the following quotation (Van Cleave, 1931; 352): "lemnisci . . . very unequal in size, the one with two giant nuclei several times larger and longer than the one bearing a single nucleus. One lemniscus often more than two-thirds the length of the body cavity."

Realization of the fact that all of the original specimens of *N. australis* came from the same host species and from the same locality strengthened the will to assume that a single, highly plastic species was involved, even though this assumption necessitated recognition of a level of individual variability more marked than had been observed previously for any member of the genus *Neoechinorhynchus.* A reexamination of the original description of *N. australis* reveals the fact that two distinct

species have been confused under the single name, *N. australis*. It therefore becomes necessary to redefine the original species and to differentiate it from a second species which is here described as *Neoechinorhynchus distractus*.

Neoechinorhynchus australis Van Cleave, 1931 (emended)
(Figs. 2, 4, 5, 6, 8, 11)

Accepting the originally designated female holotype of *N. australis* (accession no. VC 2290.1) as the fixed point around which the emended concept of this species may be built, it becomes evident that the paratype male originally designated as allotype (VC 2293.5) is not conspecific with the holotype. Therefore a new allotype (VC 2293.7) is selected from among the five paratypes conforming to the emended concept of *N. australis*.

Description.—With the characteristics of the genus *Neoechinorhynchus* as expressed by Meyer 1932. Body relatively small, short and thick, without conspicuous dimorphism of the sexes, although no fully gravid female has been observed. Two observed males 3.4 and 6.5 mm long by 0.38 to 0.42 mm in maximum diameter. Observed females 3.6 to 10.7 mm long by 0.34 to 0.67 mm in maximum diameter. The two lemnisci showing marked disparity in size (Figs. 2, 4), the one with two nuclei at least twice the length of and much wider than the one with a single nucleus. The longer often extending approximately two-thirds the length of the trunk. Genital organs of male (Figs. 5. 6) extending through about one-half of the body cavity and the long lemniscus reaching backward beyond the level of the anterior testis. Testes broad almost filling width of body cavity, their ends in broad contact. Heavy, syncytical cement gland about same diameter as the testes, in contact with posterior testis.

Proboscis (Figs. 2, 4) elongate-globular. 146 to 230 μ long by 117 to 170 μ in diameter; armed with three circles of six hooks each. Hooks of terminal circle usually 88 to 90 μ long; middle circle usually about 47 to 59 μ; basal circle 23 to 35 μ.

No fully formed hard shelled embryos observed.

Type host: *Ictiobus* sp., at Money, Mississippi.

Holotype female (VC 2290.1) and five paratypes, of which one male (VC 2293.7) is designated as allotype, are in the collection of H. J. Van Cleave at Urbana, Illinois. The former allotype of *N. australis* (VC 2293.5) becomes allotype of *N. distractus* while one of the former paratypes of *N. australis* (VC 2288.2) is herein designated as holotype of *N. distractus*.

Meyer (1932: 73) expressed the belief that *N. australis* as originally defined is very close to *N. cylindratus*. This belief is founded on an inadequate appreciation of the distinctiveness of *N. australis*, either as originally described or as emended in this article. Even a cursory glance at specimens of these two species dispels any inclination to regard them as more closely related than is expressed in their being included within the same genus. The diversity of the two lemnisci in *N. australis* is not fortuitous or lacking in taxonomic significance for it reflects a distinct line of differentiation which is held in common by several species. This distinction alone throws *N. australis* into sharp contrast with *N. cylindratus*. Furthermore, the hooks of the middle circle on the proboscis are much longer in *N. australis* than in *N. cylindratus*.

N. australis (as emended) may be distinguished from other species of the genus which show great diversity in size of the two lemnisci within the same individual, in the following points: 1. The body of *N. australis* (Fig. 5) is distinctly shorter than that of *N. distractus* (Fig. 7). 2. The proboscis is larger in *N. australis* than in *N. distractus*. 3. The male genitalia occupy more than half of the body cavity in *N. australis* (Figs. 5, 6) while in *N. distractus* (Fig. 7) they occupy considerably

less than half the length of the cavity. 4. Correlated with extent of the male genitalia, the longer lemniscus in *N. australis* (Figs. 5, 6) extends beyond the anterior margin of the front testis while in *N. distractus* (Fig. 7) the lemnisci end far anterior to the testes. 5. The terminal hooks of *N. australis* are distinctly larger than in *N. venustus* and in *N. cristatus*. 6. The dorsal body wall of *N. australis* lacks the extreme thickening characteristic for *N. cristatus*.

Neoechinorhynchus distractus n. sp.
(Figs. 1, 3, 7, 9, 10)

Synonym: *N. australis* Van Cleave, 1931, in part.

With the characteristics of the genus *Neoechinorhynchus*. Body relatively long and slender, of males (Fig. 1) 5.9 to 7.3 mm long by 0.35 to 0.38 mm in maximum diameter; of observed females (Fig. 3) 8.4 to 19.6 mm long by 0.38 to 0.67 mm in maximum diameter. Proboscis elongate-globular, 140 to 176 μ long by 120 to 164 μ in diameter, most often about 146 μ long by 123 μ in diameter. Hooks of terminal circle usually 59 μ long, of middle circle 41 μ, of basal circle 23 to 29 μ.

The two lemnisci in the same individual (Figs. 1, 3) extremely diverse in size. In males (Fig. 3) the long lemniscus (containing two giant nuclei) ends far anterior to the anterior testis. In allotype male the testes are long, cylindrical, with the ends somewhat pointed, much narrower than the width of the body cavity, with considerable distance between the two testes. Syncytial cement gland not in contact with posterior testis, narrow and much smaller than width of body cavity.

No fully mature hard shelled embryos observed but some in which the outer shell was not fully formed measured 26 to 35 μ long by 15 μ broad.

Type host, *Ictiobus* sp., at Money, Mississippi.

Holotype female (VC 2228.2) and 8 paratypes, of which one male (VC 2293.5) is designed as allotype, in collection of H. J. Van Cleave, Urbana, Illinois. The allotype of this species was originally designated as allotype of *N. australis* while the holotype and paratypes of *N. distractus* were originally confused with *N. australis* and were designated as paratypes of that species.

Following is a summary of the points available for distinguishing between *N. distractus* and other members of the genus that seem to be closely related to it.

1. *N. distractus* differs from *N. australis* in having distinctly smaller hooks in the terminal (59 vs. 88 μ) and middle (41 vs. 47 to 59 μ) circles of the proboscis hooks. The males of *N. distractus* have the gonads far removed posteriorly from the free end of the long lemniscus. From species other than *N. australis* which have conspicuous diversification of the two lemnisci, *N. distractus* may be distinguished as follows: 2. The terminal hooks of *N. distractus* average larger than those of *N. cristatus* (59 vs. 45 to 59 μ). 3. The dorsal body wall of *N. distractus* does not show the conspicuous thickening characteristic of *N. cristatus*. 4. In males of *N. distractus* there is a long space between the free end of the long lemniscus and the front testis while in both *N. cristatus* and *N. venustus* the end of the long lemniscus overlaps the front testis. 5. As corollary of this statement, the gonads in males of *N. cristatus* and *N. venustus* extend much farther toward the front end of the body than in *N. distractus*.

Neoechinorhynchus strigosus, n. sp.
(Figs. 12 to 20)

Diagnosis: With the characters of the genus *Neoechinorhynchus*. Marked sexual dimorphism, the males relatively small and weak, the gonads apparently undergoing early degeneration and length of life apparently much shorter than females, body lacking anterior attenua-

tion characteristic of female. Mature females (Fig. 16) 9 to 14.1 mm long, trunk elongate cylindrical, without conspicuous inflation in any region, posterior extremity diminishing rather rapidly in size, anterior extremity usually with a relatively long, narrowed region tapering to a diameter but slightly wider than that of the neck; the attenuated portion usually bent ventrally in a conspicuously rounded curve of short radius. Maximum diameter of trunk usually 500 to about 700 μ. Proboscis distinctly globular, with a diameter much more nearly equal to that of the front end of the trunk than in most members of the genus, 105 to 146 μ in length by 132 to 158 μ (most frequently 146 μ) in width. Hooks of terminal circle usually about 58 μ long (53–64 μ); middle circle usually about 41 μ (32 to 53 μ); basal circle 29 to 38 μ.

Observed males 3.7 to 5.5 mm in length, lacking the conspicuous anterior attenuation and ventral bending characteristic of the female. Maximum diameter about 346 μ. Proboscis about 105 μ long by 146 μ in width. Hooks slightly smaller than in females. Genital organs of male normally occupying slightly more than 0.6 of the length of the trunk. Testes elongate, usually contiguous, relatively small, diameter considerably less than diameter of the body cavity. Cement gland elongate, narrow, usually considerable distance posterior to the posterior testis. In several males the testes have undergone partial or extensive histolytical break-down, leading in one instance to a monorchid condition and in another to complete loss of distinct testes, leaving but a few scattered fragments in their places.

Embryos within preserved gravid female considerably shriveled, lacking perfectly formed outer membranes; 53 to 72 μ long by 26 to 31 μ wide.

Hosts: *Ictiobus bubalus* of Reelfoot Lake, Tennessee; *Catostomus commersonnii* of Lake Chetac, Sawyer County, Wisconsin; and *Moxostoma aureolum* from the Mississippi River in western Wisconsin.

Types: Holotype and series of paratypes in the collection of H. J. Van Cleave, Urbana, Illinois.

The elongately cylindrical body of *N. strigosus* is distinctively different from that of most species of the genus except *N. distractus* and from the latter *N. strigosus* is easily distinguished by the fact that the lemnisci in *N. strigosus* are not conspicuously different in size and in the females the anterior end of the trunk is usually attenuated.

The terminal organ (PO, Fig. 18) located centrally within the anterior extremity of the proboscis in *N. strigosus* is much more conspicuous than in most members of the genus. The third, fourth, and fifth dorsal giant nuclei of the subcuticula are distinctively different from the form usually found in members of this genus. There is but rarely any elevation of the body surface to mark their location. They are so long and narrow (Fig. 15) that they rarely cause any thickening of the body wall in the region where they are located. Often individual nuclei reach a length of approximately 1 mm.

In some individuals, the attenuated anterior end of the trunk shows evidence of unusual capacity for longitudinal contraction. At times (Fig. 14) this results in formation of a thickened, localized band but in other instances it may be less pronounced and merely produce a series of closely set narrow plications (P, Fig. 14) encircling the body. In one series of individuals from *Moxostoma aureolum* the bodies showed conspicuous alterations which seemed to be due to treatment before or duration fixation. The anterior dorsal giant nuclei of the subcuticula were in prominent papilla-like projections (N I, N II Fig. 13) of the body wall. In these same individuals the body wall was often ruptured in the vicinity of the second dorsal nucleus allowing the lemnisci or at times the anterior testis to protrude through the wall (TA, Fig. 13). In some instances the break was through the ventral wall and in others through the dorsal wall.

The general shape of *N. strigosus* is distinctive, especially in the female (Fig. 16) with its slimly cylindrical body attenuated and arched in the anterior region of the trunk. The narrowly elongate body of the male (Figs. 12, 13) has the reproductive organs reaching farther anteriorly than in any other species of the genus with similar body form. The hooks of the second circle on the proboscis are not conspicuously smaller than those of the first circle as maintains for other species of the genus.

Along with the high incidence of speciation which has been attained by the CATOSTOMIDAE on this continent several equally distinctive species of NEOECHINORHYNCHIDAE are found, maintaining either characteristic or exclusive host relations with these fishes. *N. strigosus* and *N. distractus,* which are recognized for the first time in the present paper, take their place with the following typical NEOECHINORHYNCHIDAE of the catostomid fishes: *Octospinifer macilentus* Van Cleave, *Neoechinorhynchus crassus* Van Cleave, *N. australis* Van Cleave, *N. venustus* Lynch and *N. cristatus* Lynch, bringing to seven the species of NEOECHINORHYNCHIDAE characteristic of North American CATOSTOMIDAE.

Information regarding the species of NEOECHINORHYNCHIDAE from catostomid fishes in North America is too fragmentary to permit of final analysis of the geographical distribution of the species. However, from facts now available (Table I) it is indicated that *Neoechinorhynchus cristatus* and *N. venustus* are distinctive of the northwestern part of the United States with the only records from the state of Washington. *N. australis* and *N. distractus* have been recorded from the state of Mississippi only. *N. strigosus* occurs in various hosts of the north central states from Tennessee and Wisconsin within the Mississippi River drainage. *N. crassus* has attained wide dispersal in the Great Lakes area, extending from Ohio and Michigan westward to Washington. *Octospinifer macilentus* is distinctively eastern and northern in distribution with records from Connecticut and New York westward into Michigan, Wisconsin, Illinois and Ontario.

TABLE I.—*Records of the occurrence of* Neoechinorhynchidae *in the* Catostomidae *of North America, compiled from the literature and from the collections of the writer.*

Species	Hosts	Localities
Neoechinorhynchus australis Van Cleave	*Ictiobus sp.*	Tallahatchie R., Miss.
N. crassus Van Cleave	*Catostomus commersonnii*	Douglas L., Mich.; Lakes and streams, Wis. Lake Erie; Waskesin L., Saskatchewan; L. Washington, Wash.
	Erimyzon oblongus	L. Pepin, Minn.
	Erimyzon sucetta	Buckeye L., O.
	Moxostoma erythrurum	Lakes and streams, Wis.
	Moxostoma rubreques	Streams, Wis.
N. cristatus Lynch	*Catostomus macrocheilus*	L. Washington, Wash.
N. distractus Van Cleave	*Ictiobus sp.*	Tallahatchie R., Miss.
N. strigosus Van Cleave	*Ictiobus bubalus*	Reelfoot L., Tenn.
	Catostomus commersonnii	L. Chetac, Wis.
	Moxostoma aureolum	Mississippi R., Wis.
N. venustus Lynch	*Catostomus macrocheilus*	L. Washington, Wash.
Octospinifer macilentus Van Cleave	*Catostomus commersonnii*	Douglas L., Mich.; Oneida L., N. Y.; Lake Erie; Dixon L., Algonquin Park, Ontario; Lakes and streams, Wis.
	Erimyzon oblongus	Conn.
	Carpiodes carpio	Rock R., Ill.

Life cycles are unknown for all of these parasites but it seems probable that similar food habits within the CATOSTOMIDAE may explain the adaptations of the parasites to their definitive hosts since there is no extensive evidence of strict host specificity.

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EXPLANATION OF PLATES

All drawings were made with the aid of a camera lucida from stained permanent mounts. Katharine Hill Paul, scientific artist in the department of Zoology in the University of Illinois, prepared all the drawings and arranged the plates.

Symbols

B —copulatory bursa
C —copulatory cap
C G —cement gland
F —funnel of uterus
L 1 —uninucleate lemniscus
L 2 —binucleate lemniscus
N V —ventral subcuticular nucleus
N I, N II, etc.—dorsal subcuticular nuclei numbered from anterior end
P —plications of body wall
P O —terminal organ of prosboscis
R —cement reservoir
S —selective apparatus
S D —dorsal ligament sac
S V —ventral ligament sac
T A —anterior testis
T P —posterior testis

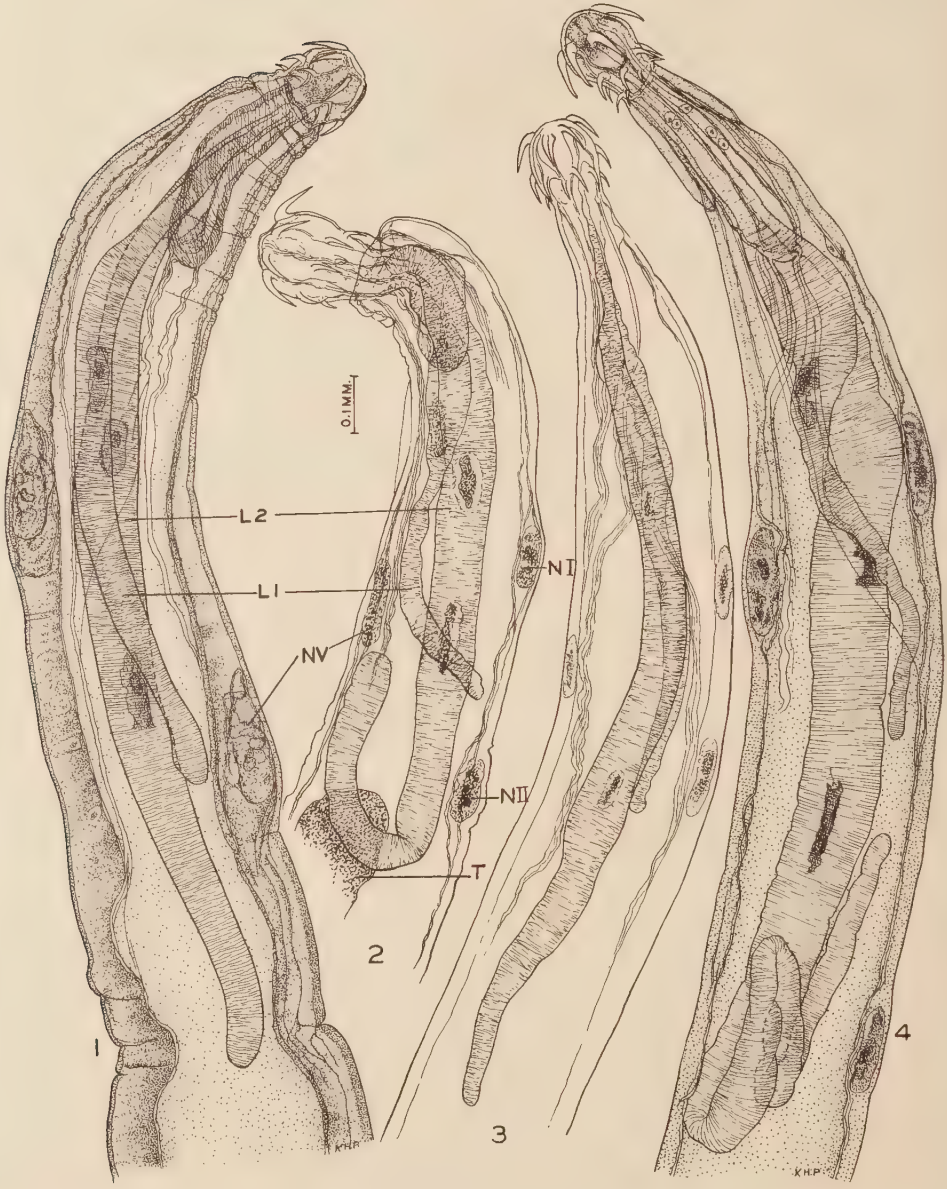

PLATE I

Size dimorphism in the lemnisci of *Neoechinorhynchus australis* and *N. distractus*, n. sp. All figures were drawn to the same scale, the line indicating magnification is 0.1 mm long.

FIG. 1. Anterior extremity of the body of holotype female of *N. distractus*.

FIG. 2. Anterior extremity of body of a male of *N. australis*.

FIG. 3. Anterior extremity of body of a male of *N. distractus*.

FIG. 4. Anterior extremity of body of holotype female of *N. australis*.

PLATE II

Arrangement of organs of *N. australis* and *N. distractus*.

FIGS. 5 to 9 drawn to the same scale.

FIGS. 5 and 6. Comparison of fully grown (Fig. 5) and an immature male (Fig. 6) of *N. australis* showing extent of the lemnisci and their relations to the genital organs.

FIG. 7. Morphology of allotype male of *N. distractus*, showing wide separation of the testes from the lemnisci.

FIG. 8. General morphology of holotype female of *N. australis*.

FIG. 9. Anterior extremity of holotype female of *N. distractus*, showing limitation of the longer lemniscus (L 2) to the level anterior to the second dorsal giant nucleus of the subcuticula (N II).

FIG. 10. Posterior extremity of a female of *N. distractus*, showing copulatory cap (C) attached to the narrowed genital extremity.

FIG. 11. Posterior extremity of an immature female of *N. australis*, showing the funnel of the uterus (F) opening into the ventral ligament sac (S V).

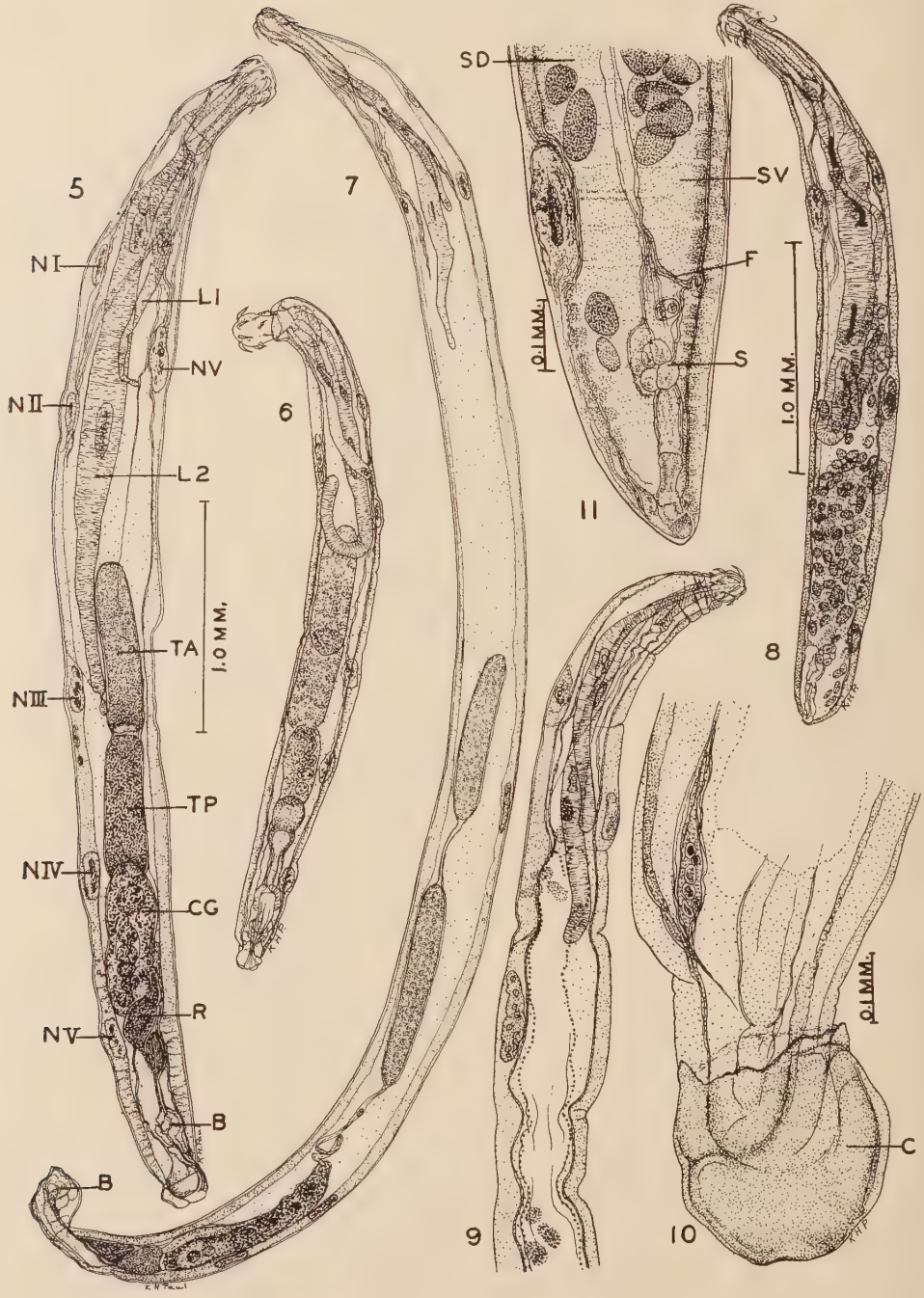
5
7
6
11
8
9
10
NI
LI
NV
NII
L2
TA
NIII
TP
NIV
CG
R
NV
B
B
SD
SV
F
S
0.1 MM.
1.0 MM.
1.0 MM.
0.1 MM.
C

PLATE II

Plate III

Morphology of *N. strigosus,* n. sp.

The scale with Fig. 12 applies also to Fig. 14, that with Fig. 20 applies to Figs. 17 to 20 inclusive.

Fig. 12. Male from *Catostomus commersonnii,* showing arrangement of genital organs.

Fig. 13. A male from *Moxostoma aureolum* showing characteristic rupture of the body wall, probably produced by osmotic changes at the time of fixation.

Fig. 14. Anterior extremity of female showing effects of contraction in producing prominent swelling of trunk with surface plications (P).

Fig. 15. A segment of the trunk in region of the third dorsal subcuticular nucleus, showing elongation of the nucleus in the body wall.

Fig. 16. Outline drawing of a mature female showing characteristic body form. Embryos filling ligament sacs are shown conventionally by stippling.

Figs. 17 and 18. Proboscis of *N. strigosus* from *Ictiobus bubalus.*

Fig. 19. Proboscis of *N. strigosus* from *Catostomus commersonnii.*

Fig. 20. Posterior extremity of trunk of a female of *N. strigosus.*

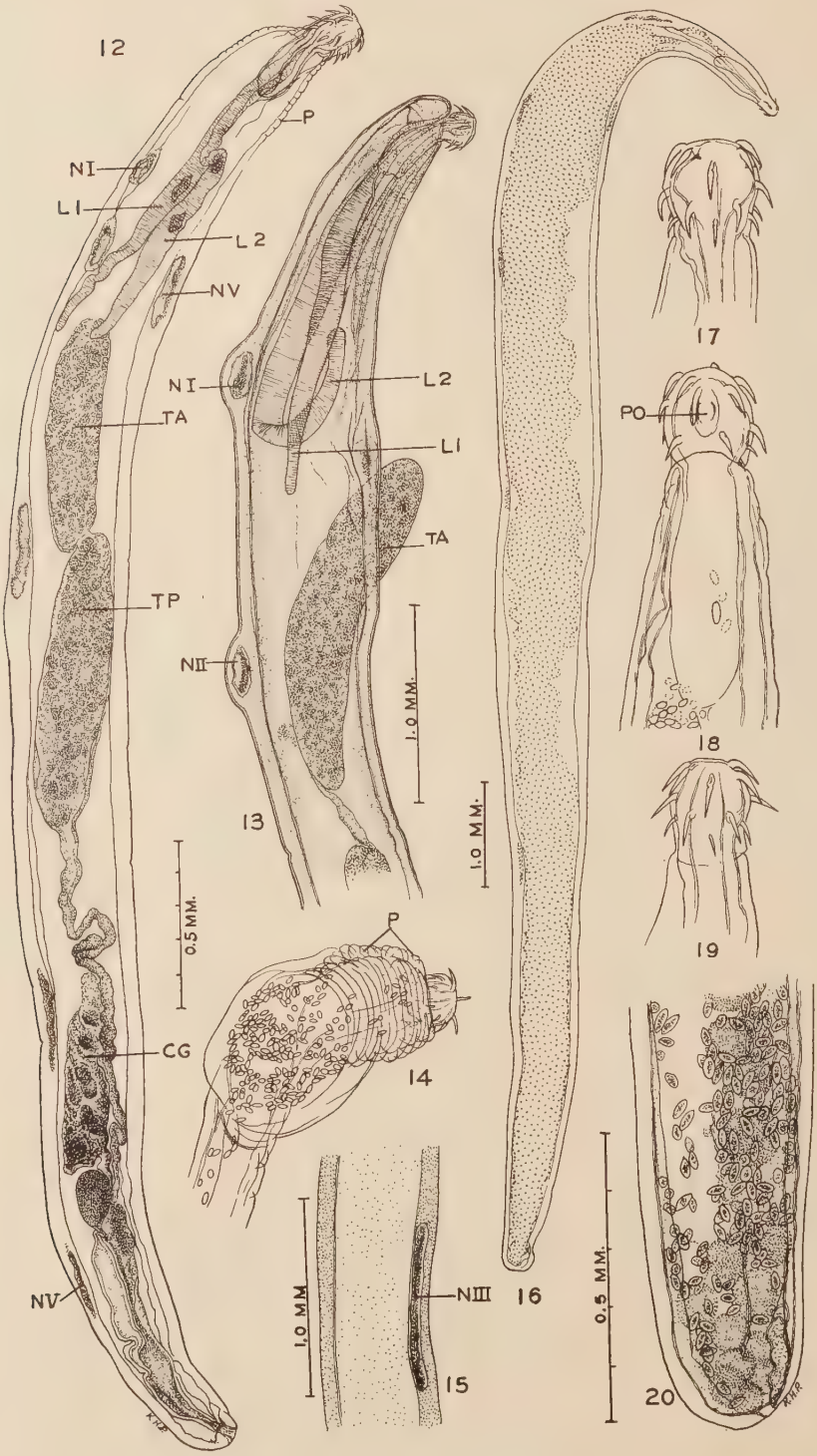
12
P
NI
L1
L2
NV
TA
TP
NV
CG
0.5 MM.
13
NI
L2
L1
TA
NII
1.0 MM.
14
P
15
1.0 MM
NIII
16
1.0 MM.
17
PO
18
19
20
0.5 MM.

PLATE III

THE LIFE HISTORY OF *POSTHARMOSTOMUM HELICIS* (LEIDY, 1847) n. comb. (TREMATODA: BRACHYLAEMIDAE)

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INTRODUCTION

This study was begun during the spring of 1946, after the discovery of metacercariae, subsequently identified as *Postharmostomum helicis* (Leidy, 1847), in the pericardial cavities of the pulmonate snails *Polygyra thyroidus* and *Anguispira alternata* collected in Rockland County, New York. Several hundred *A. alternata*, some of which contained metacercariae and four of which were shedding brachylaemid cercariae (later proved to be of the same species as the metacercariae) were collected in the same region during the next two years. These naturally infected snails provided the larval flukes used to establish the infection in experimental animals, and their laboratory raised progeny were used as trematode-free invertebrate hosts in later experiments.

Adult flukes, recovered from mice to which the metacercariae had been fed, were found to agree in morphological details with the diagnosis of the subfamily BRACHYLAEMINAE as given by Allison (1943) This subfamily was included in the family HARMOSTOMIDAE, established by Odhner (1912), with *Harmonostomum* Braun, 1899 as the type genus. Joyeux and Foley (1930) stated that *Harmostomum* Braun, 1899 is a synonym of *Brachylaima* Dujardin, 1843, corrected orthographically to *Brachylaemus* by Blanchard (1847), and that *Brachylaemus* is a valid genus. They changed the name of the family, therefore, to Brachylaemidae. Allison (1943) reviewed the literature on the morphology and taxonomic relations of members of the family.

The life cycles of nine species of the BRACHYLAEMINAE have been traced experimentally, and portions of others are known. Life histories have been experimentally determined for *Distomum leptostomum* (= type of genus *Harmostomum* Braun, 1899, = *B. leptostomum*), by Blochmann (1892) and Hofmann (1899); *Harmostomum horisawai,* by Ozaki (1925); *Brachylaemus fuscatus,* by Joyeux, Baer and Timon-David (1932a, 1932b, 1934); *B. virginiana, Glaphyrostomum mcintoshi* and *Panopistus pricei,* by Krull (1935a, 1935b, 1935c); an unnamed species of *Brachylaemus,* by Dollfus, Callot and Desporte (1935); *Postharmostomum gallinum,* by Alicata (1940); and *Leucochloridiomorpha constantiae,* by Allison (1943). Life histories of several species of *Leucochloridium,* belonging to another subfamily of the BRACHYLAEMIDAE, have been elucidated. Work on species of this genus was reviewed by Robinson (1947) in a report on the life cycle of *Leucochlor-*

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Gratitude is expressed also to Doctor William Clench for identifying specimens of *A. alternata, Z. arboreus* and *D. reticulatum* used in this work.

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idium fuscostriatum. Sinitsin (1931) described parts of several brachylaemid life cycles as deduced from morphological similarity of stages found in naturally infected animals, but unsupported by experimental corroboration. Balozet (1937) described stages of the development of *B. suis* in naturally infected snails and experimentally infected definitive hosts, but was unable to infect various species of snails by feeding them eggs of the fluke.

Ulmer (1949) and Robinson (1949), almost simultaneously, published preliminary descriptions of the life cycle of *P. helicis.* Ulmer used the name *Postharmostomum laruei* for the fluke, and neither writer was aware of the duplication of effort until the publication of these papers, but it is evident that both did essentially similar work on the same species.

The adult trematode described in the present paper agrees morphologically and is regarded as specifically identical with the fluke described and named *Postharmostomum laruei* by McIntosh (1934). McIntosh reported this trematode as occurring naturally in the cecum of the chipmunk, *Tamias striatus lysteri.* He fed metacercariae from land snails to white rats and recovered adult worms identical with those found in the chipmunk. McIntosh indicated that in classifying the worm in the genus *Postharmostomum* he was not finally deciding upon the validity of the genus. Miller (1939) also recovered adult flukes of this species from rats fed metacercariae from undesignated species of *Polygyra* and *Anguispira.*

According to McIntosh (1934), the genus *Postharmostomum* was erected by Witenberg in 1923. Witenberg (1925) subdivided the genus *Harmostomum* Braun, 1899 into two subgenera: *Harmostomum* new subgenus and *Postharmostomum* Witenberg, 1923, and listed the distinguishing features of each subgenus. His list of references did not include Witenberg (1923), which was cited in the text.

Since then, other writers (e.g., Alicata, 1940) have considered *Postharmostomum* to be of generic rank. In Witenberg's (1925) classification, the feature which best distinguishes *Postharmostomum* from *Harmostomum* is the winding course of the ceca. The writer believes that the regular and constant sinuosity of the ceca is a characteristic which requires the acceptance of *Postharmostomum* as a genus, and agrees with McIntosh's assignment of the fluke from the chipmunk to this genus.

In all essential features, the metacercarial stage of the trematode, whose life cycle is reported here, agrees with the description given by Leidy (1847) for a distomate fluke from the pericardial cavity of *Helix alternata* Say, 1816 (= *Anguispira alternata* (Say) Tryon, 1866) which he named *Distoma helicis.* Creplin (1849), apparently without justification, renamed this fluke *D. pericardium.* Diesing (1855), without giving reasons for the change, listed *D. helicis* Leidy, 1847 under the name *Cercariaeum Helicis alternatae,* with the name given by Leidy as a synonym.

Leidy (1850) described and figured developmental stages of a larval brachylaemid which he named *D. vagans.* As noted by McIntosh (1934), this description apparently included individuals of more than one species. The first and second stages described for *D. vagans* appear to be young forms of the metacercariae previously described by Leidy (1847) as *D. helicis,* and accordingly may be identified with that species. The third stage described for *D. vagans,* characterized by ciliated excretory ducts and a different location in the host, may be regarded as typical of the species for which the name *D. vagans* is reserved.

There are references in the literature (e.g., Hofmann, 1899; Witenberg, 1925; Joyeux, Baer and Timon-David, 1934; Allison, 1943) to a brachylaemid metacercaria supposed to have been described and named *Distoma helicis* by Meckel in 1846. Had Meckel used this specific name in 1846, it would not have been available for the larva described by Leidy in 1847. Although Meckel (1846) briefly described and figured a brachylaemid metacercaria in this paper, he did not name the fluke, and extended search has not disclosed any other communication in which the name *D. helicis* was proposed by Meckel. Indeed, Diesing (1850) proposed the name *Distoma helicis pomatiae* for the fluke described, but presumably unnamed, by Meckel (1846); and later, Diesing (1855) transferred this species to *Cercariaeum.* Leidy's use of the specific name *D. helicis* was therefore valid, and that name is the correct one for the trematode to which he applied it.

The apparent identity of *D. helicis* Leidy, 1847 and the metacercaria described in the present paper, and the morphological similarity of the adult flukes described by McIntosh (1934) and those described here, makes it likely that the metacercariae used by McIntosh in his experiments were of the same species as those described by Leidy in 1847. McIntosh made no reference to *D. helicis,* and apparently was unaware that a larval stage of the fluke had already been named when he proposed the name *Postharmostomum laruei* for the brachylaemid from the chipmunk. It appears, therefore, that the name of the fluke the life cycle of which is reported in this paper is *Postharmostomum helicis* (Leidy, 1847) n. comb., and that *D. pericardium, C. Helicis alternatae* and *P. laruei* are synonyms.

MATERIALS AND METHODS

The striped wood snail, *Anguispira alternata,* is the first and usual second intermediate host of *Postharmostomum helicis.* These and other snails, collected during the course of the work reported here, were maintained in terraria and fed lettuce. The snails reproduced readily in the laboratory, and many of the progeny of the wild snails were raised for use in infection experiments.

Larvae from wild snails were used for study and infection experiments on the vertebrate and invertebrate hosts, until cercariae and metacercariae from laboratory raised, experimentally infected gastropods were available. Dissections showed that about five per cent of the *A. alternata* harbored metacercariae of *P. helicis* when brought into the laboratory. A year later, about seventy-five per cent of the snails were parasitized by from one to fifteen metacercariae as the result of continuous exposure to cercariae from two of the four naturally infected, shedding snails which were kept in the stock terrarium. The remaining two of these snails were kept isolated from each other and from other snails harboring sporocysts.

The deer mouse, *Peromyscus leucopus,* was the experimental final host first used successfully in the laboratory. A female with blind, suckling young was caught, and two of the young were reared for use as experimental hosts. These mice were kept for sixteen days before being used in infection experiments, and daily examinations showed that their feces did not contain trematode eggs until more than fourteen days after experimental exposure. Since in subsequent infection experiments the vertebrate hosts first passed eggs about twenty-one days after exposure, it may be assumed that the young mice did not contain *P. helicis* when brought into the laboratory. The female deer mouse was not parasitized by tre-

matodes, as shown by fecal examinations and autopsy. Both black and white laboratory mice also were used as definite hosts, but the flukes would develop only in very young mice, and few eggs were recovered from them.

Ten metacercariae from naturally infected *A. alternata* were fed to each of the two young deer mice with a pipette, care being taken that the larvae were swallowed and not expelled. At an undetermined time between two and four weeks later, both mice began passing trematode eggs.

Eggs from one of the deer mice were fed to laboratory raised *A. alternata* and *P. thyroidus,* and to two species of slugs, *Philomycus carolinianus* and *Deroceras reticulatum* Müller. Only those snails which passed eggs or egg shells in their feces were considered to have been exposed. Some of the *A. alternata* were dissected at weekly intervals to obtain developmental stages of the sporocysts; the infection was allowed to develop to maturity in the remaining specimens. Four infected snails were kept isolated from each other and from all other snails parasitized by sporocysts. The other species of mollusks did not contract the infection.

Laboratory raised *A. alternata, P. thyroidus* and *Zonitoides arboreus,* as well as wild *P. carolinianus* and *D. reticulatum,* were exposed to cercariae from the experimentally infected snails, and upon subsequent dissection developing or mature metacercariae were found in specimens of all five species. Other laboratory raised *A. alternata* and *P. thyroidus* were exposed to cercariae from naturally infected snails; after a minimum time of ten weeks both species contained infective metacercariae.

Adult *P. helicis* for study were obtained from one of the deer mice which died forty-nine days after exposure, and from laboratory mice sacrificed from eight to one hundred fifty days after exposure to metacercariae from naturally and experimentally infected snails. The second deer mouse was still passing eggs seven months after it had been exposed to infective larvae.

Normally, the eggs hatch only in the intestine of *A. alternata.* Miracidia for study were obtained by putting eggs which had been passed at least two months previously into tap or distilled water. Possibly due to an osmotic effect, the operecula of some of the eggs came loose and the miracidia were expelled. This was not normal hatching, as the miracidia did not move during or after the process, and the emergence of the miracidium was almost instantaneous.

Sporocysts from both natural and experimental infections were studied in whole mounts and in serial sections of the hosts' digestive glands. Cercariae for study were obtained from both naturally and experimentally infected *A. alternata* by washing them from the surface of the snails with a fine stream of water. Metacercariae from both experimentally and naturally infected mollusks were studied in whole mounts and in sections of them within the hosts. Corresponding larval stages of the fluke from experimentally and naturally infected snails were morphologically identical.

Developmental stages of the adult fluke were obtained from laboratory mice, and sexually mature worms were recovered from laboratory mice and a deer mouse. The mature flukes were studied in whole mounts and in serial sections.

Most of the material studied in whole mounts was stained with Mayer's paracarmine; some was stained with Ehrlich's acid hematoxylin. All sections were stained with Harris' hematoxylin and eosin.

THE LIFE CYCLE: EXPERIMENTS AND OBSERVATIONS

The eggs of *P. helicis* contain fully developed miracidia (Fig. 2) when passed from the mouse, and these miracidia are immediately infective to *A. alternata*. Eggs used in infecting the first intermediate host were in most cases passed during the preceding twenty-four hours. Although infective, the miracidia usually are motionless within the egg; movement was seen but once in hundreds of eggs examined. In this instance, the anterior end of the larva was pushed against the operculum by a rhythmical beating of the cilia which also rotated the miracidium in one direction at a rate of 180 revolutions per minute. Miracidia were not stimulated to movement by juices from macerated snail intestine during the thirty minutes they were observed.

In infection experiments involving the first intermediate host, twenty-three laboratory raised and five wild *A. alternata,* two laboratory raised and one wild *P. thyroidus,* and one specimen each of *D. reticulatum* and *P. carolinianus* were exposed to eggs of *P. helicis*. Of the laboratory raised *A. alternata,* sixteen became infected, and those in which the infection was allowed to mature began shedding cercariae eleven to sixteen weeks after exposure. Cercariae first appeared on most infected snails eighty to eighty-eight days after exposure to the eggs. One snail harbored sporocysts containing only germ balls thirteen weeks from the time it had been fed the fluke eggs, and it is not known how much longer it would have taken for the infection to develop to maturity.

Seven of the successfully exposed *A. alternata* were dissected in unsuccessful attempts to find sporocysts less than two weeks old. In these seven snails, infections might have been present but undetected.

Of the wild *A. alternata,* four started to shed cercariae between eleven and thirteen weeks after exposure, and one which was only lightly exposed did not become infected.

Very few cercariae can be washed from a snail with a newly mature infection, but about one month later dozens of cercariae are produced each day. A snail can shed cercariae for at least nine months. Sporocysts were found in very small, half grown and fully mature *A. alternata* which had been experimentally exposed to eggs.

In the laboratory, the snails will eat mouse feces, and usually their intestines contain a considerable amount of soil. Since the eggs hatch only in the intestine of *A. alternata,* it is apparent that in nature these snails become infected with sporocysts after eating eggs of *P. helicis,* either in rodent feces or in ingested soil.

The youngest sporocyst found (Fig. 4) was seen in serial sections of the digestive gland of an *A. alternata* killed fifteen days after exposure to the fluke eggs. Nothing was learned, therefore, of the biology and morphology of the trematode during its first two weeks in the snail. Since most young sporocysts, including the one fifteen days old, were found in the digestive gland at some distance from the intestine, a migration of the young larva occurs during this time. After fifteen days in the snail the sporocyst is a hollow structure containing loose cells, and lies in a lacuna in the connective tissue of the dorsal, anterior part of the host's digestive gland.

At thirty-one days of age the sporocyst (Fig. 5) has grown considerably, and shows the first indications of branching. The cavity contains loose cells and a few

clumps of from two to sixteen cells. The wall consists of a thick cuticula with from one to several layers of cells on the inner side.

Growth after thirty days is rapid. The sporocyst branches in several directions, many germ balls develop within the cavity and the wall becomes thinner in some places. The more posterior parts of the digestive gland are penetrated by long branches beneath its investing membrane, from which shorter branches grow and penetrate the substance of the gland and subdivide. By the time the sporocyst is mature, parts of it may be found in hemal spaces, the connective tissue of the kidney, fatty connective tissue between the host's organs, and even in the lumen of the heart. Secretory cells of the digestive gland are not directly penetrated, but are damaged and eventually destroyed by some more indirect means, possibly pressure or lysins. The fatty connective tissue likewise is destroyed, but no damage was noted to the epithelial cells of the kidney, hemal spaces and intestine, or to the membrane surrounding the digestive gland. One month after cercariae first are shed, almost the entire digestive gland, as well as parts of the rest of the snail, may contain sporocyst tissue.

Little was learned of the germinal material which ultimately produces the cercariae. The fifteen day old sporocyst contains loose cells, presumably germinal in nature, as do the older sporocysts. In later stages of development there are also germ balls, developing cercariae and fully developed larvae in the sporocysts. None of these cells or larvae invariably are limited to a particular part of the sporocyst, although some branches may contain only germ balls or mature cercariae. The origin of the presumably germinal cells was not discovered, nor the length of time they function.

No evidence for the production of a daughter generation of sporocysts was found. After five and one-half weeks of development there are many spherical germ balls in the cavities of the sporocysts, and by the eighth week some of them are recognizable as developing cercariae. Sporocysts older than eight weeks contain germ balls, immature cercariae and fully developed cercariae. In some sporocysts seven and eight weeks old there are a few large, solid bodies (Fig. 9) with no constant size or shape, whose nature was not determined. Their lack of organization makes it unlikely that they are daughter sporocysts.

In the development of germ balls into cercariae, the first step is a slight elongation of the spherical mass into an oval one. Then some of the cells become organized into the rudiments of the suckers and the pharynx. At almost the same time the pre-pharynx and esophagus, and then the short ceca, become visible. While these changes are occurring, some of the cells posterior to the ventral sucker form a dense aggregation which eventually assumes the shape of the germinal primordium. The tail is not differentiated at first, but as the embryo becomes more and more elongated, a small group of cells becomes distinctly delimited as the tail.

At first, no motility is shown by the larva, but after all the structures have become differentiated and the approximate final size and shape have been reached, the cercaria begins to move. Mature cercariae are capable of crawling through the hollow branches from one part of the sporocyst to another.

Cercariae leave the sporocyst by means of birth pores located at the ends of some of the branches, and migrate to the surface of the snail. Gross dissections of three infected snails and serial sections of two others showed that their hemal

spaces contained many free cercariae. The mantle cavity also contained cercariae, but none were found in the lumen of the digestive gland. The cercariae are capable of penetrating solid tissue, as shown by the fact that they have been found in the foot and other parts of the snail. It appears, therefore, that after the cercariae leave the sporocyst they migrate by way of hemal spaces to the region of the mantle, through the thin tissue of this organ into the mantle cavity, and thence to the outside through the respiratory pore.

All of nine *A. alternata* dissected from eighteen hours to sixteen weeks after exposure to cercariae of *P. helicis* contained metacercariae in various stages of development. Mature metacercariae were found in small, half-grown and full grown *A. alternata.* The larvae develop either in the pericardial cavity or the kidney of the host, the pericardial cavity being the usual location. The migration of the cercariae into these cavities has not been observed by the writer, but experiments showed that the process takes less than eighteen hours. In several other brachylaemid species, the cercariae enter the pericardial cavity from the surface of the snail by way of the kidney duct, kidney and reno-pericardial connection (Sinitsin, 1931; Joyeux, Baer and Timon-David, 1934; Alicata, 1940). The speed with which the larvae of *P. helicis* reach their final site in the second intermediate host suggests that this may be the route followed.

Although *A. alternata* is the usual second intermediate host of *P. helicis,* other gastropods may serve. One *P. thyroidus* was found naturally infected, and three laboratory raised specimens were experimentally infected with metacercariae. One wild *P. carolinianus* was exposed to cercariae, and upon dissection thirty-three days later, larval flukes, identical with others experimentally obtained from *A. alternata,* were recovered from the pericardial cavity. It is possible that this infection was a natural one, but in any case the larvae appeared to be those of *P. helicis.* A wild *D. reticulatum* and a laboratory raised specimen of *Z. arboreus* were exposed to cercariae from an experimentally infected snail; three days later each contained very young larvae, with ceca extending just posterior to the ventral sucker. The ceca of mature metacercariae (Fig. 11) are convoluted and their ends are near the excretory bladder; the short ceca, as well as the small size, of the worms recovered in this experiment indicated that they had parasitized the second intermediate host for a very short time, and had resulted from the experimental exposure of the mollusks three days earlier.

There is evidence that cercariae of *P. helicis* shed from a snail cannot re-enter the same snail to become metacercariae. The two *A. alternata* with naturally acquired sporocysts which had been in close association with each other for at least nine months contained one and twelve metacercariae, respectively, when dissected. The other two snails with natural infections and the four laboratory raised *A. alternata* harboring experimentally produced sporocysts, all six of which had been kept isolated, were dissected weeks or months after cercariae first were shed from them. None of the six contained living metacercariae, although one had a dead, flattened and hardened metacercaria in its kidney. If self-infection is possible, it is likely that all eight of these sporocyst-bearing snails would have contained a large number of larvae, as snails with no sporocysts usually harbored from twenty to eighty metacercariae after experimental exposure to cercariae. Since the two *A. alternata* which had been exposed to cercariae shed by each other did not contain an unusual

number of metacercariae, and the six snails which had been exposed only to cercariae shed by sporocysts which they themselves harbored contained none, it seems probable that self-infection is unusual or impossible.

The dead larva in the kidney of one snail suggests that there may be an immunity mechanism in the snails which eventually kills any cercariae which accidentally re-enter the snail from which they were shed. If such an immunity is present, it operates only in the situation described above, for experiments have shown that snails harboring sporocysts subsequently can become parasitized by metacercariae, presumably after exposure to cercariae shed from other snails, and snails containing metacercariae can at a later time become infected with sporocysts.

The cercariae are active after they reach the pericardial cavity, and move about and feed while attached to the heart or the walls of the pericardium. No open lesions were observed in sections of hearts of infected snails, but the activities of the parasite cause the endothelial covering of the heart to undergo a noticeable hyperplasia and hypertrophy, until it is heavily papillated and in some places hangs in shreds. In spite of the apparent absence of open lesions, the presence of solid cellular material in the ceca of the larvae indicates that they probably feed upon the heart tissue.

The cercarial tail persists for about ten weeks after the worms have reached the pericardial cavity of the second intermediate host; its loss may be taken to indicate the transformation to the metacercarial stage. Four *A. alternata* and one *P. thyroidus* dissected from seven to fifty-one days after exposure to cercariae contained tailed, non-infective larvae. One *A. alternata* dissected sixty-three days after exposure harbored numerous larvae, about half of which had tails while the rest did not; those without tails were somewhat narrower than infective larvae, and did not develop further when fed to a young white mouse. An *A. alternata* dissected sixty-seven days after experimental exposure to cercariae contained thirty-one larvae, twenty-five of them without tails and mature in appearance. One with a tail was full grown; the other five tailed larvae were of different sizes, none of them as large as mature metacercariae. The twenty-six full grown larvae were fed to a young white mouse, but did not produce an infection. Neither this nor the preceding experiment can be considered conclusive, as even young laboratory mice sometimes were recalcitrant to infection.

Of three snails dissected eighty-two days after exposure, an *A. alternata* and a *P. thyroidus* contained infective metacercariae, while another *A. alternata* had twenty-eight small, immature larvae in its pericardial cavity. A *P. thyroidus* and an *A. alternata* dissected ninety-one and one hundred twelve days, respectively, after exposure contained only mature metacercariae.

The evidence from these experiments suggests that most of the larvae of *P. helicis* become mature in appearance after nine to ten weeks in the second intermediate host; that some develop at a considerably slower rate than others; and that possibly after attaining final size and shape they must go through a period of physiological maturation before becoming infective to the final host.

The infective metacercariae are much less active than the younger larvae, although they do not become encysted. They readily fall from the pericardial cavity when the host is dissected, indicating that they are attached feebly or not at all. Sections of several snails containing metacercariae also show that many of them lie

free in the pericardial cavity. On the other hand, actively feeding larvae cling to the heart, even against the suction of a pipette.

The chipmunk, which has been reported (McIntosh, 1934) as a final host of *P. helicis* in nature, eats some animal matter, and often lives in or near rockpiles and dead logs inhabited by colonies of *A. alternata*. The metacercariae are relatively inactive, and there is no exit from the pericardial cavity large enough for their passage. These facts show that chipmunks become infected by eating snails containing the infective stage of the trematode.

In experiments on the definitive host, two deer mice and twenty-six laboratory mice were exposed to metacercariae; the deer mice and eleven of the laboratory mice became infected. Of these eleven laboratory mice, nine were less than half grown when exposed. Fifteen laboratory mice did not become infected after exposure. Two of these mice were fed metacercariae which possibly were immature, in experiments described above; thirteen were exposed to larvae believed to be fully infective. Six of these thirteen mice which did not become infected were fully grown when exposed, and three were half grown. The remaining four were very young. An age immunity is indicated by these facts, since the successfully infected laboratory mice generally fell in a much younger age group than those which did not become infected.

None of the infected laboratory mice passed as many eggs as did the deer mice, and no eggs ever were found in the feces of some of the mice from which adult worms were recovered at autopsy. Infection was not produced in a hamster, pigeon, chicken, common mole, cottontail rabbit and English sparrow which were fed metacercariae. All of these animals except the sparrow and chicken were adults.

The eggs of *P. helicis* appear in the feces of the host after about twenty-one days. They were found on the twenty-first and twenty-second day after exposure, respectively, in the feces of a white and a black laboratory mouse, but so few eggs were passed that it is possible that some were in the feces previous to these times but were not detected. Neither deer mouse was passing eggs fourteen days after exposure, but both were passing eggs in large numbers on the twenty-eighth day. No examinations were made in the interval.

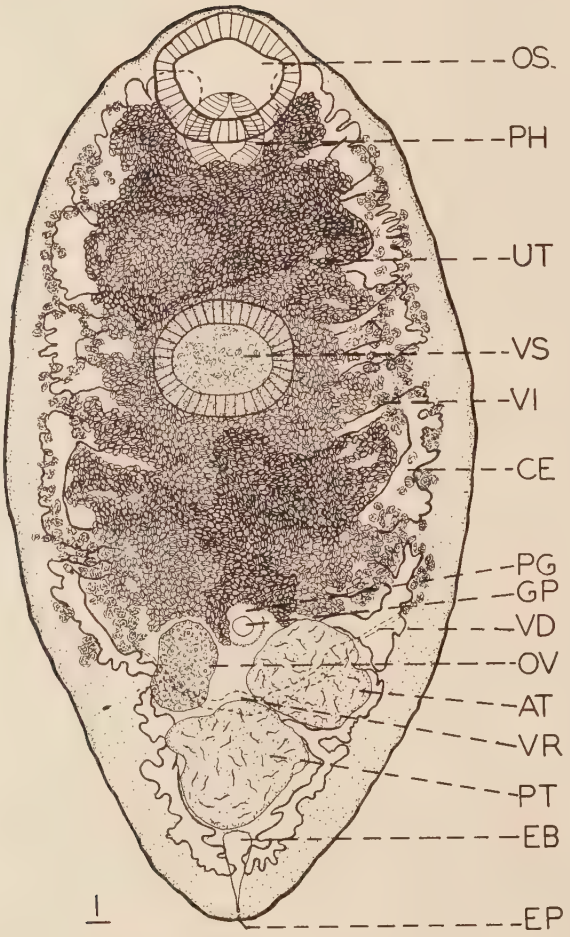
The flukes develop at a somewhat variable rate after reaching the final host. One worm from a mouse autopsied thirteen days after exposure had fairly extensive vitellaria and several dozen eggs in the uterus, while another had almost no vitellaria and contained no eggs. One of three sexually mature flukes from another mouse was only two-thirds the size of the other two, although all three were of the same age and from the same location in the host.

The adult *P. helicis* live in the cecum of the mouse host, often at the blind end. Their activities cause hemorrhages visible on the outer surface of the cecum, and the fecal material in these areas is bloody. When they are removed from the mouse their ceca contain blood corpuscles and other material which is quickly expelled. The largest number of *P. helicis* recovered from a mouse in these experiments was forty-two.

Morphology of the Stages

The egg (*Fig. 2*)

The egg of *P. helicis* measures 31 to 37 microns long (av. 34.8) and 14.5 to 18 microns broad (av. 17.5), and is roughly oval, with one side more convex than the other. The shell is yellowish and moderately heavy, and frequently there is a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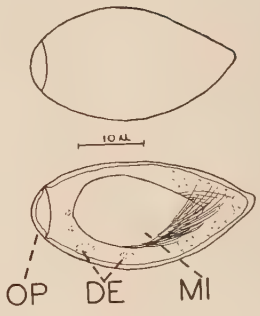
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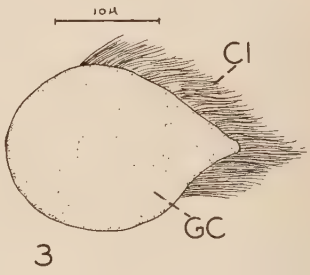
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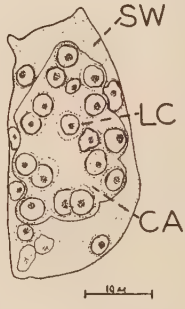
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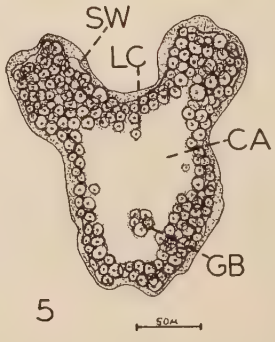
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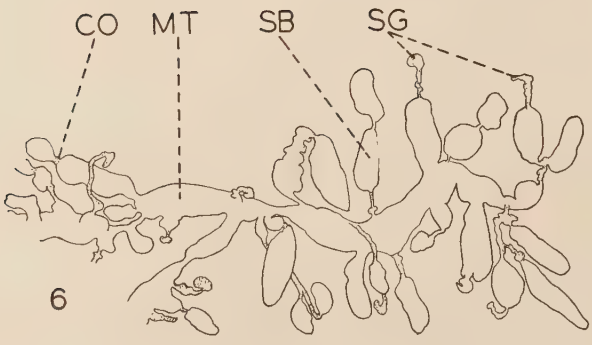
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thickening at or near the adopercular end. The contained miracidium usually is motionless; the cilia on its posterior end and the granular detritus between it and the shell are easily visible.

The miracidium (Fig. 3)

The miracidium is a partially ciliated larva 21 microns long and 10 microns broad, wider in the anterior half and pointed posteriorly. The cilia, about 10 microns long, are arranged along the posterior half of the presumably dorsal midline, around the posterior tip and on the most posterior part of the ventral midline. Limited distribution of the cilia is characteristic of the miracidia of brachylaemid flukes. The internal morphology was not studied, but several refractile cells are distinguishable in various parts of the unstained larvae.

The sporocyst

After fifteen days of development in the snail the sporocyst (Fig. 4) is a hollow, oval structure 40 microns long and 25 broad. The distribution of the cell nuclei in the walls indicates that the sides are one cell thick and the ends several cells thick. In the one specimen studied there was a difference between the two ends, one having about twice the thickness of the other.

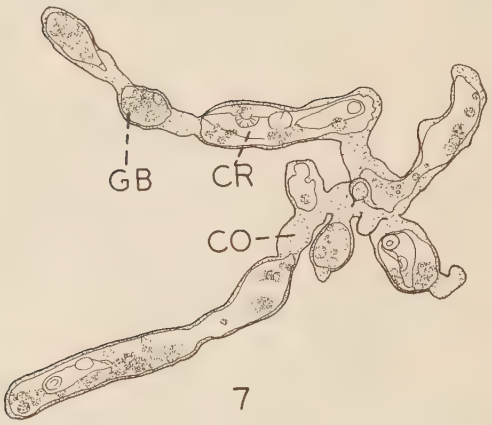
The cavity of the sporocyst contains about thirty cells up to 6.5 microns in diameter, including a few protruding from the wall. These cells are characterized by their small amount of cytoplasm, an even, heavy deposit of chromatic material on the inside of the nuclear membrane, and a large, usually central nucleolus. Similar cells can be discerned in the sporocyst wall, but their nuclei do not stain as heavily. Presumably, the cells in the cavity of the sporocyst are germinal in nature, but no study was made concerning their fate.

After thirty-one days of growth the sporocyst (Fig. 5) is 200 to 300 microns in diameter, irregular in shape and in some cases has short projections which are the beginnings of branches. The wall consists of an outer cuticula and several layers of cells; most of these cells have large nuclei and only a small amount of cytoplasm, and are similar in appearance to those found in the cavity of the fifteen day old sporocyst. This type of cell also is found loose in the cavity and in young germ balls.

At thirty-eight days the sporocyst is much branched, and in the wider branches there are small, spherical germ balls in large numbers. Some of the larger branches have thin walls made up of a cuticula and a layer of cells; other branches have walls several cells in thickness, with nuclei similar to those found in the walls and cavity of the fifteen day old sporocyst.

At fifty-six days the sporocyst is more extensively branched and contains many germ balls up to 30 microns in diameter, and an occasional large, elongated or oval mass (Fig. 9). These masses are solid, the nuclei of their cells appear to be pyknotic, the entire mass is deeply staining except for occasional areas containing no

FIG. 1. Adult *P. helicis* from deer mouse; camera lucida.
FIG. 2. Eggs of *P. helicis*, one showing contents; free-hand.
FIG. 3. Miracidium, swollen by distilled water; free-hand.
FIG. 4. Fifteen-day old sporocyst, median sagittal section; camera lucida.
FIG. 5. Thirty-one-day old sporocyst, section; camera lucida.
FIG. 6. Portion of living, mature sporocyst; free-hand.



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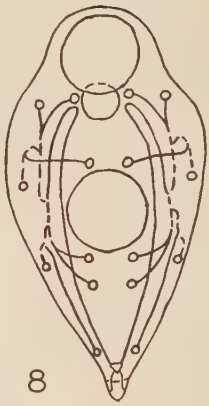

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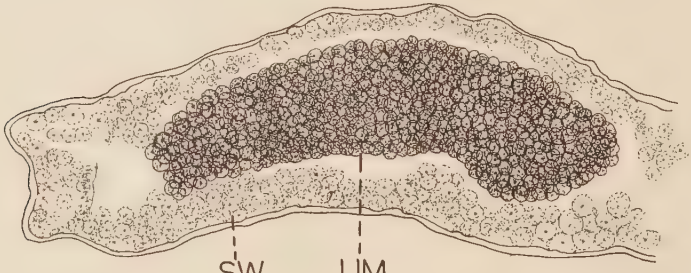

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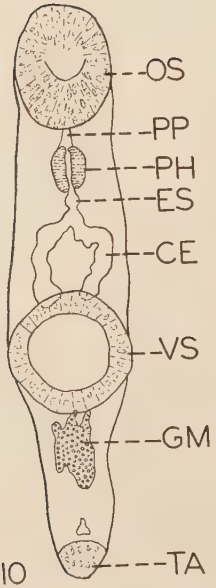

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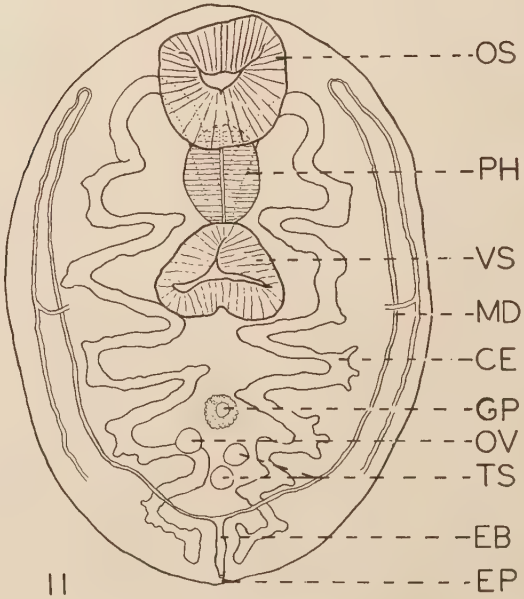

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cells, and there is no discernible structure or constant shape. In size they measure up to at least 115 microns in length and 75 microns in width. The walls of the sporocysts vary from one to several cells in thickness (Fig. 9), and many of the nuclei in the cells are of the distinctive type described in the younger sporocysts. In their organization and appearance, some of the smaller branches look, in section, like the sporocysts at thirty-one days of age.

The mature sporocyst (Figs. 6, 7) is a branched system of tubes the lumen of which is separated into various-sized cavities by irregularly spaced constrictions, through which the cavities are continuous by narrow channels. The tubes are branched and rebranched at variable intervals, and often several branches grow from a main trunk in a limited area. When alive, the sporocyst is colorless and immotile. The maximum width of a fixed and stained sporocyst branch, either sectioned or whole, is about 200 microns, and branches containing no larvae may be less than 40 microns wide. Most of the branches are between 75 and 125 microns in width. The constrictions are from 20 to 50 microns wide.

In most places, the wall of the sporocyst consists of a cuticula and a single layer of cells, and is from 3 to more than 10 microns in thickness. Around the birth pores at the ends of some branches the wall is several cells thick. No flame cells were seen in the live sporocysts studied.

Developing larvae are found in any part of the sporocyst. A cavity between constrictions may contain from one to twelve or more cercariae, as well as loose cells and spherical germ balls. Some of the cavities contain only germ balls, or one or a few fully-developed cercariae.

The cercaria (Fig. 10)

The cercaria of *P. helicis* is a generally elongate, flattened, stump-tailed larva with a prominent pharynx and no stylet. The suckers are large, and the ventral sucker is posterior to the center of the body. There are widely scattered spines on the cuticula, probably sensory in function. The pre-pharynx and esophagus are short and visible only in extended specimens, and the ceca extend to the level of the ventral sucker.

Measurements of fifteen representative, fully developed, extended specimens taken from a crushed snail are as follows: length, 238 to 331 microns (av. 279); width, 54 to 90 microns (av. 74); oral sucker size, 58 to 68 microns in length and 50 to 58 microns in width (av. 62 by 56 microns); ventral sucker size, 54 to 61 microns in length and 50 to 72 microns in width (av. 55 by 67 microns); germinal

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Fig. 7. Portion of mature, stained sporocyst, whole mount; camera lucida.

Fig. 8. Diagram of cercarial excretory pattern; ducts shown in broken lines not seen.

Fig. 9. Unidentified body in seven-week-old sporocyst, section; camera lucida.

Fig. 10. Cercaria, whole mount; camera lucida.

Fig. 11. Metacercaria, whole mount; camera lucida. Gonads semi-diagrammatic.

Abbreviations: AT, anterior testis; CA, sporocyst cavity; CE, cecum; CI, cilia; CO, constriction; CR, cercaria; DE, detritus; EB, excretory bladder; EP, excretory pore; ES, esophagus; GB, germ ball; GC, granular cell; GM, germinal primordium; GP, genital pore; LC, loose cell; MD, main excretory collecting duct; MI, miracidium; MT, main trunk; OP, operculum; OS, oral sucker; OV, ovary; PG, prostate gland; PH, pharynx; PP, pre-pharynx; PT, posterior testis; SB, side branch; SG growth points; SW, sporocyst wall; TA, tail; TS, testes; UM unidentified mass; UT, uterus; VD, vitelline duct; VI, vitellaria; VR, vitelline reservoir; VS, ventral sucker.

primordium size, 22 to 36 microns in length and 22 to 40 microns in width (av. 30 by 29 microns). When contracted, the cercariae are wider anteriorly and considerably shorter.

There are eight pairs of flame cells in the excretory system (fig. 8), each quarter of the worm having two lateral and two medial cells. All connections of the ducts from the flame cells were not determined, but probably the ducts of each pair join, the two ducts thus formed in each quadrant connect to form one duct draining the quadrant, and on each side these join to form the main collecting duct. The main collecting ducts pass from the bladder forward near the sides of the larva to the level of the pharynx, then posteriorly to the level of the ventral sucker, where they are formed by the ducts from the quadrants. The bladder is roughly spherical when full, and empties by way of a pair of ducts through two lateral pores, one on each side of the tail. In the body of the cercaria the ducts from the bladder are broad, but in the tail they are very narrow except when wastes are passing through. According to Allison (1943), the tissue between these ducts in the tail of *L. constantiae* is homologous to the "island of Cort" in strigeid cercariae, and presumably the same is true in *P. helicis*.

In stained specimens a germinal primordium, not visible in living larvae, is evident just behind the ventral sucker. It is irregular in shape, and may be partially divided longitudinally into three parts.

In the living larvae, two aggregations of an undetermined number of glands are visible lying posterior and lateral to the ventral sucker, with their ducts leading to a small area at the anterior edge of the oral sucker.

The metacercaria (Fig. 11)

The mature, live metacercaria is a grayish, translucent, broadly oval, flattened, ventrally concave worm. When first removed from the snail it moves sluggishly, but in a short time becomes motionless and whitish in appearance. It is capable of attaching to objects with its suckers. The pharynx is conspicuous, and the prepharynx and esophagus are visible only in extended specimens. The ceca follow a convoluted course to the posterior end of the body, usually with six, sometimes with seven medial loops. The cuticula is thick, and in worms not under pressure it is wrinkled to give a ridge-like or scale-like appearance. The main collecting ducts of the excretory system are formed by subsidiary ducts at the level of the ventral sucker and follow the same course as in the cercaria; they lie lateral to the ceca along most of their length, but pass ventral to them to reach the excretory bladder. The excretory pore is slightly subterminal and dorsal in living specimens, but seems to be terminal in some of those which have been fixed.

Measurements of fifteen representative, morphologically mature specimens fixed under moderate pressure but somewhat contracted are as follows: length, 735 to 1,020 microns (av. 802); width 510 to 690 microns (av. 585); oral sucker size, 155 to 216 microns in length and 155 to 198 microns in width (av. 166 by 182 microns); ventral sucker size, 101 to 126 microns in length and 133 to 209 microns in width (av. 116 by 163 microns); pharynx size, 76 to 126 microns in length and 86 to 119 microns in width (av. 99 by 102 microns). Characteristically, the ventral sucker contracts more strongly longitudinally than transversely when the worm is fixed; the oral sucker usually is roughly circular.

The genital pore lies in the ventral midline about halfway between the ventral sucker and the posterior end. Reproductive system rudiments are visible to varying degrees in stained specimens. A deeply stained group of cells always is present around the genital pore; presumably, this mass is the rudiment of the prostate gland and other male organs. A more elongated and indefinite structure usually is visible just posterior to the genital pore, in the region of the female accessory reproductive organs in the adult fluke. The rudiments of gonads are faintly visible in favorable specimens.

The adult (Fig. 1)

The living adult *P. helicis* is whitish, except for the brown eggs in the uterus and the reddish contents of the ceca. It is broad, flat and sluggish in its movements, becoming motionless almost immediately in tap water.

The cuticula is smooth, the oral sucker is subterminal, the ventral sucker is just anterior to the center of the long axis of the worm, the pharynx is prominent, and the ceca are convoluted, usually with six medial loops, have small outpocketings when empty, and extend almost to the posterior end of the worm.

The gonads are arranged in a triangle behind the ventral sucker, with the ovary on the right side, the posterior testis in the center and the anterior testis on the left. The ovary is oval and usually indented on two sides; the anterior end of this organ is somewhat closer to the median plane of the worm than the posterior end. The testes are irregularly shaped and indented on all edges. The posterior testis usually is wider than long. In worms from the deer mouse the gonads may touch or actually overlap; in those from laboratory mice the gonads are relatively smaller and do not touch each other at any point. In all specimens, the zone of the ovary lies in the zone of the anterior testis and may overlap that of the posterior testis; the zone of the anterior testis may or may not overlap the zone of the posterior testis; and the fields of the ovary and anterior testis both overlap that of the posterior testis, but do not overlap each other.

In the male reproductive system, the efferent duct of the posterior testis follows a course from the center of the anterior face of the gonad to a region just posterior or dorsal to the genital pore, and almost touches the medial surface of the ovary along most of its course. The duct from the anterior testis starts from the dorsal part of the antero-medial surface of the gonad and runs to the median plane of the worm. Here the two male ducts join, and continue as the vas deferens. For most of its length this duct is wide, thin walled and looped, and is anterior to the genital pore. This wide portion is filled with a thin, non-cellular substance, but in two sectioned specimens no spermatozoa were observed. The egg masses obscure the region in whole mounts. The terminal portion of the vas deferens becomes narrow and muscular, and is surrounded by the prostate gland. It then enters the cirrus sac. The cirrus is short and unarmed.

The vitellaria are follicular and sparse, and they extend from the level of the pharynx to that of the ovary. Two vitelline ducts pass dorsal to the ovary and anterior testis but ventral to the ceca, and meet in the median plane at the level of the posterior end of the ovary to form a prominent vitelline reservoir. A common vitelline duct leads dextrally, ventrally and anteriorly from the vitelline reservoir.

Laurer's canal is present, opening at the surface of the body on the left of the midline, at or near the level of the posterior edge of the anterior testis. The proximal and distal portions of the canal are straight, thick-walled and narrow; the central portion is looped, thin-walled and considerably wider. The loops are approximately dorsal to the vitelline reservoir, and contain cellular debris, some of it identifiable as vitelline material.

The oviduct leaves the medial face of the ovary by way of a fecundarium, and leads toward the midplane of the fluke. It loops dorso-ventrally soon after leaving the ovary; Laurer's canal joins the oviduct in this region. The common vitelline duct then joins the oviduct, just as it turns anteriorly. The duct thus formed constitutes the oötype; this structure is about twice the diameter of the oviduct when empty. Spermatozoa are found in the oviduct, from the ovary to the region of the oötype, and in the proximal portions of the two canals which join it. None were seen in the initial portion of the uterus.

The uterus runs anteriorly from the oötype, and is thin-walled and at first relatively narrow. It soon turns and follows a sinuous course toward the anterior end of the ovary, then becomes broader and contains newly formed eggs. Both the ascending and descending arms of the uterus wind from side to side, and in worms from the deer mouse are for the most part indistinguishable from each other because of the contained mass of eggs. Specimens from laboratory mice contained fewer eggs, and the ascending arm of the uterus on the right side and the descending arm on the left can be seen to wind extensively and irregularly, both dorsoventrally and from side to side. The pharynx is flanked by two loops of the uterus; the body of the fluke between this level and the genital pore, and between the medial edges of the ceca, is filled with uterine loops. The last portion of the descending arm, distinguished by the very dark eggs it contains, usually lies in the dorsal part of the median plane of the worm, from the level of the ventral sucker to the genital pore. Usually this part of the uterus is somewhat convoluted, and occasionally it does not lie entirely in the median plane. Near the genital pore the uterus becomes narrow and thick-walled, then widens to form a muscular metraterm which ends at the genital pore; both of these last regions of the uterus are surrounded by gland cells.

The main collecting ducts of the excretory system are lateral to the ceca for most of their length, but pass ventral to the ceca to reach the bladder. The excretory bladder when collapsed extends anteriorly almost to the posterior testis.

Measurements of twenty worms are given in table I, because there are certain differences in the proportions of flukes taken from the two final host species.

Three differences are outstanding. The total size of worms from the deer mouse was greater than of those from the laboratory mice, but so many factors which were not controlled in these experiments may affect the size that the variation is not necessarily important. The other two differences, the relatively smaller gonad size and ventral sucker size of specimens from the laboratory mice, may be of significance.

In the flukes from the deer mouse, the oral sucker averaged 1.03 times the size of the ventral sucker in both dimensions; in ten sexually mature worms from laboratory mice, the length of the oral sucker averaged 1.3 times that of the ventral sucker, and the width averaged 1.4 times the width of the ventral sucker. In other specimens recovered from laboratory mice but not included in table I, either inspec-

TABLE I

Fluke Number*	Time in Host	Length	Width	Oral Sucker	Pharynx	Ventral Sucker	Anterior Testis	Posterior Testis	Ovary
1	49 da.	3.6 mm.	1.88 mm.	425 × 615 mi.	300 × 315 mi.	450 × 645 mi.	450 × 480 mi.	495 × 555 mi.	360 × 255 mi.
2	"	3.75	1.65	480 × 495	255 × 255	465 × 510	495 × 525	375 × 390	345 × 225
3	"	3.7	1.8	510 × 600	300 × 300	465 × 600	510 × 465	345 × 600	330 × 270
Av.		3.68	1.78	472 × 570	285 × 290	460 × 585	485 × 487	405 × 515	345 × 250
4	9 da.			210 × 255		188 × 180			
5	"			240 × 270		203 × 165			
6	"			285 × 240		165 × 165			
7	"			240 × 285		180 × 180			
8	"			240 × 270		180 × 195			
Av.				243 × 264		183 × 177			
9	13 da.			285 × 360		255 × 285			
10	"			240 × 285		225 × 218			
Av.				262 × 322		240 × 251			
11	21 da.	3.24	1.62	450 × 600	215 × 240	383 × 420	360 × 345	285 × 390	270 × 225
12	"	2.76	1.3	435 × 540	255 × 240	360 × 450	285 × 240	210 × 285	225 × 180
13	"	3.15	1.5	480 × 570	195 × 233	420 × 435	315 × 330	315 × 360	300 × 165
14	"	3.0	1.5	420 × 600	240 × 255	450 × 495	300 × 270	300 × 420	270 × 240
15	"	2.63	1.23	405 × 495	215 × 180	330 × 420	285 × 315	285 × 270	300 × 165
Av.		3.0	1.43	438 × 561	224 × 230	389 × 444	309 × 300	279 × 345	275 × 195
16	49 da.	2.27	0.98	420 × 450	180 × 195	330 × 375	195 × 165	195 × 240	195 × 150
17	"	2.72	1.13	465 × 525		420 × 390	225 × 225	225 × 285	195 × 165
Av.		2.5	1.05	445 × 488		375 × 383	210 × 195	210 × 263	195 × 158
18	83 da.	3.72	1.72	525 × 525	255 × 270	325 × 480	310 × 325	345 × 525	315 × 195
19	"	3.6	1.72	540 × 570	285 × 285	340 × 450	480 × 315	325 × 340	300 × 240
20	"	3.4	1.56	555 × 570	240 × 255	325 × 480	345 × 325	315 × 435	270 × 210
Av.		3.57	1.67	540 × 555	260 × 270	330 × 470	378 × 322	328 × 433	295 × 215
Av. of nos. 11–20 (sexually mature)		3.0	1.38	474 × 535	242 × 250	365 × 432	299 × 272	272 × 347	255 × 189
Measurements from McIntosh (1934)		2.8	1.4	410 × 500	250 × 250	420 × 450	290 × 200	185 × 290	250 × 197

* Flukes 1–3 from deer mouse. Flukes 4–20 from laboratory mice. Flukes 4–10 immature.

tion or measurement showed the ventral sucker to be noticeably smaller than the oral sucker. The sucker sizes of immature flukes, numbers four to ten in the table, show the same phenomenon.

The mature worms from the deer mouse were 1.23 times the length and 1.29 times the width of those from the laboratory mice, but the ovary, anterior testis and posterior testis of the specimens from the deer mouse were, respectively, 1.35, 1.6 and 1.5 times as long as the corresponding organs in the ten worms from the laboratory mice, and 1.32, 1.8 and 1.48 times as wide. In this case, too, inspection of many specimens from laboratory mice showed that the gonads were noticeably smaller, in relation to the total size of the flukes, than those from the deer mouse.

The worms here described which grew to maturity in laboratory mice are very similar to the diagnosis given by McIntosh (1934) for *P. laruei,* except that their gonads are somewhat larger and ventral suckers smaller. The worms from the deer mouse have considerably larger gonads than the specimens described by McIntosh, and have more eggs in the uterus. These differences probably are not specific, but are due to varying effects to different hosts on the flukes, and to individual variation.

DISCUSSION

The life cycle of *P. helicis* follows the pattern reported for the nine species in the subfamily previously determined experimentally. Typically, in all these life cycles the miracidium emerges from the egg in the gut of a pulmonate snail, enters mesenchymatous tissue, and develops into a branched sporocyst. The sporocysts produce stump-tailed cercariae which migrate to the surface of the snail; then in some cases re-enter the same snail and in others penetrate another snail of the same or a different species. In specific organs of the second intermediate host the cercariae become metacercariae, usually unencysted. The final host is infected by eating snails containing these metacercariae. Exceptions to this scheme are *Leucochloridiomorpha constantiae,* presumably a primitive brachylaemid, which utilizes a branchiate snail for its first and second intermediate hosts and has a fork-tailed cercaria (Allison, 1943), and *Glaphyrostomum mcintoshi,* in which the cercariae do not leave the sporocyst (Krull, 1935b).

Experimental work here reported shows that five species of mollusks are acceptable second intermediate hosts of *P. helicis,* and Ulmer (1949) listed additional species. In this characteristic *P. helicis* is like other brachylaemid flukes, for in at least five of the experimentally determined life cycles from three to nine species of mollusks were listed as second intermediate hosts for a brachylaemid species.

Since attempts to infect *P. thyroidus, D. reticulatum* and *P. carolinianus* with miracidia were unsuccessful, and since Ulmer reported that he could not infect other species of *Polygyra,* it is not likely that gastropods other than *A. alternata* will be found to serve as the first intermediate host of *P. helicis.* One brachylaemid, *Panopistus pricei,* is known to utilize either a snail or a slug as its first intermediate host (Reynolds, 1938), but this condition has not been reported for other species.

The approximate time for the completion of one life cycle of *P. helicis* is twenty-four weeks, but this time probably will vary with the temperature and other environmental conditions in which the snail hosts are kept. Although Ulmer (1949) reported that the metacercariae were not yet infective after eighteen weeks, in other respects the time required for development of the successive stages he gave are

similar to those reported here. Ulmer did not describe the environment of his experimental snails, so it is not known whether different conditions of temperature explain the different rates of development reported for the metacercarial stage.

The time necessary for development of the various stages, particularly those in the snail, is appreciably longer in *P. helicis* than has been reported for other brachylaemids. For example, Krull (1935a) found that the entire cycle of *B. virginiana* is completed in seventy days, and the same writer (Krull, 1935c) reported that development of the two stages of *P. pricei* in the snail hosts was completed in a total of fifty-eight days. In *P. gallinum,* these two stages are completed in a minimum of one hundred fifteen days (Alicata, 1940). No reason is apparent for the unusually long time required for the development of *P. helicis* in the successive hosts.

Individual differences in the rate of development of all stages were reported above. These differences are explicable in several ways, none of them proved by experiments. The rate of development of sporocysts probably is influenced by factors in the individual hosts, the varying amounts of food and moisture available to the snails, and the temperature in which they live. These same conditions might influence the development of metacercariae in the second intermediate host, and it also is possible that some cercariae are older and hence more developed than others, and thus become infective after a shorter period of growth. This latter condition may affect the rate of development of the adult worm; it may be that newly infective metacercariae must undergo longer development in the rodent host to reach maturity than those which have been in the snail host for a long period of time after reaching the infective stage. In the case of worms from the same location in the same host, the environment must be essentially the same for all, and other conditions are responsible for variations noted. Interesting as the problem may be, no explanation of the facts is available.

The worms from laboratory mice ranged from two-sevenths to one and five-sevenths times the age of those from the deer mouse, and included two identical in age. Therefore, age differences do not account for the relatively larger ventral suckers and gonads of the specimens from the deer mice. Variations of characteristics in trematodes from different host species are not unknown, and if the differences noted in these few specimens prove to be constant among a large number, then *P. helicis* furnishes another example of the phenomenon. The facts also indicate that laboratory mice are less suitable hosts than deer mice.

Several writers have discussed the possibility of cercariae re-entering the snail from which they were shed to become metacercariae. They do so in *B. fuscatus,* according to Joyeux, Baer and Timon-David (1934); in *P. gallinum,* according to Alicata (1940); in *B. suis,* according to Balozet (1937); and in *B. virginiana,* according to Krull (1935a). They do not in *P. pricei,* according to Krull (1935c). There is strong, although not yet conclusive, evidence that in *P. helicis* the metacercariae cannot develop to maturity in the snail from which they were shed. No explanation of this fact is available, nor of specific differences in this respect, even within the same genus.

It has not been determined experimentally whether any species of brachylaemid trematode develops a daughter generation of sporocysts. Allison (1943) found elongated, unbranched sporocysts in naturally infected snails also harboring branched sporocysts, and believed the unbranched sporocysts to be a daughter gen-

eration. This stage has not been described for other members of the BRACHYLAEMIDAE.

Sinitsin (1931) reported that the brachylaemid trematodes *Entosiphonus* and *Ectosiphonus* have vermiform sporocysts, and Dollfus, Callot and Desporte (1935) described and figured oval, unbranched brachylaemid sporocysts. The writer found several unbranched sporocysts of a fluke not identified as to family in a wild *A. alternata* which also harbored brachylaemid metacercariae in the pericardial cavity. Cercariae from these sporocysts were very similar to those of *P. helicis,* except for their somewhat longer tails. Reports of unbranched brachylaemid sporocysts, including those representing a daughter generation, may be true, but are open to doubt until corroborated by experimental evidence, since sporocysts of a fluke which presumably belongs to another family and which has very similar cercariae may be present in the same species of snail or the same individual snail which harbors brachylaemid larvae.

On the other hand, no evidence was seen in the material studied which conclusively rules out the possibility of the existence of daughter sporocysts in *P. helicis.* Allison (1943) has suggested a seasonal development of a second generation of sporocysts, and if this condition is found to exist, it means that daughter sporocysts could be overlooked if present for only a short time. The nature of the large bodies in the seven and eight week old sporocysts was not determined, and they conceivably could be daughter sporocysts. The whole question remains open for all the species of BRACHYLAEMINAE, and can be resolved only by intensive study and experimentation.

SUMMARY

1. The life cycle of a brachylaemid trematode which parasitizes the snail *Anguispira alternata* as the first intermediate host, several species of mollusks as second intermediate host and two species of rodents as experimental definitive hosts is described.

2. The taxonomic position and name of the fluke are discussed. It is concluded that the correct name is *Postharmostomum helicis* (Leidy, 1847) n. comb.

3. The morphology of the successive stages in the several hosts is described.

4. Certain features of the life cycle here reported are discussed and compared to life cycles of brachylaemid flukes which already are known. It is concluded that the life history of *P. helicis* is essentially similar to those of other members of the subfamily.

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* This paper was not seen by the writer.

DESCRIPTION OF SOME SPECIES OF *RHABDOCHONA* (NEMATODA: THELAZIIDAE)[1]

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Random investigation of the helminth fauna of fresh-water fish has revealed four species of *Rhabdochona,* of which three have not been described previously. Specimens of the fourth species were assigned to *Rhabdochona cascadilla* Wigdor, 1918; errors in the original descriptions seemed to warrant a redescription.

Morphological and taxonomic work was done from specimens fixed in hot acetic-formol-alcohol and stored in 70% alcohol, with confirmation of details from living or specially mounted examples. Glycerine jelly was found to be advantageous to prepare *en face* views and for investigation of the papillae and spicules of the males. Several complete and partial serial sections were utilized. Drawings were made with aid of a camera lucida. The term "blade," used to describe a portion of the left spicule of the male, refers to the distal portion which is doubly alate. All other anatomical terminology is that used by Chitwood's *Introduction to Nematology* (1937).

DESCRIPTION OF NEW SPECIES

Rhabdochona decaturensis n. sp. (figs. 2, 9)

Specific diagnosis: Rhabdochona Railliet, 1916. Small, slender, transparent worms, attenuated at both ends. Cuticle thin, smooth, unstriated. Anterior end somewhat truncate; pseudolabia rudimentary. Stoma heavily sclerotized: prostom containing 14 minute teeth projecting anteriorly from longitudinal ridges; four minute teeth at base of prostom. Prostom cyathiform; mesostom long, narrow, of constant diameter. Esophagus very long, distinctly divided into slender anterior muscular and thick posterior glandular portions; posterior part of glandular portion vesicular and opaque. Deirids lateral, opposite midportion of stoma. Eggs embryonated when laid; ovoid; 0.031–.038 by 0.015–.023 mm; not filamented but covered with a thin gelatinous layer. Larval development in *Hexagenia.*

Female. Length 9.2–13.4 mm. Maximum width 0.093–.160 mm. Length of prostom 0.016–.025 mm; of total stoma 0.090–.110 mm; of muscular esophagus 0.217–.320 mm; of glandular esophagus 1.92–2.6 mm; of vagina 0.36–.66 mm; of tail 0.16–.23 mm. Width at base of prostom 0.033–.043 mm; at nerve ring 0.052–.065 mm; at end of esophagus 0.093–.150 mm; at vulva 0.083–.125 mm; at anus 0.036–.048 mm. Distance from anterior end to nerve ring 0.150–.170 mm; to excretory pore 0.236–.257 mm; to vulva 4.9–7.0 mm. Distance to vulva 51–58% of total body length.

Male. Length 6.8–8.0 mm. Maximum width 0.085–.10 mm. Length of prostom 0.015–.017 mm; of total stoma 0.096–.110 mm; of muscular esophagus 0.21–.27 mm; of glandular esophagus 1.70–2.0 mm; of tail 0.25–.31 mm. Width at base of prostom 0.026–.032 mm; at nerve ring 0.044–.054 mm; at end of esophagus 0.075–.096 mm; at anus 0.050–.058 mm. Distance from anterior end to nerve ring 0.140–.170 mm; to excretory pore 0.210–.250 mm. Right spicule 0.075–.10 mm long, scoop-shaped, with reflected barb near tip on left side. Left spicule 0.830–1.025 mm long, of which 0.630–.735 mm is alate. About eight pairs of preanal papillae, of which seven pairs are subventral and one pair sublateral; the sublateral pair is opposite the second subventral preanal pair; of the four most anterior subventral pairs, the right member is more anterior. Six pairs of postanal papillae, of which the second pair is sublateral, and the others subventral.

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[1] Contribution from the Department of Zoology and Physiology, University of Illinois.

[2] To Professor L. J. Thomas, under whose direction this investigation was conducted, the writer wishes to express his thanks and sincere appreciation for his advice and encouragement throughout the course of this study.

Type host: Aplodinotus grunniens.
Type locality: Lake Decatur, Illinois.
Type specimens: United States National Museum Helminthological Collection, No. 36992.

This species is described from one type and nine paratype adult females and one type and nine paratype adult males, selected from a large number of specimens encountered in the course of the study. Of 108 type hosts from Lake Decatur, only one was free from this parasite; the infections consisted of from one to over 600 worms per host, with the usual load being about 15 to 20. This species has been recovered from the same host from the Sangamon River above Lake Decatur, from the Illinois River near Grafton, Illinois, and from several specimens in fish markets in Chicago. It also has been found in a low level of incidence in about one-fifth of *Ictalurus maculatus* from Lake Decatur.

This species can be separated from all others of the genus thus far described by comparison of the length and configuration of the spicules. The left spicule of *R. decaturensis* is much longer than that of any species except *R. kidderi* Pearse, 1936. Type specimens of the latter species were borrowed for comparison; it is distinguished by a bifurcate and expanded tip of the left spicule (fig. 8); furthermore, the shaft makes up 72–76% of the entire length in *R. kidderi* and 51–56% in *R. decaturensis*. Separation from the species for which no males have been described may be accomplished by noting the entire length, except for *R. onchorhynchi* Fujita, 1940; this species has a vagina only 0.05 mm long, only a third as long as that of *R. decaturensis*, probably reflecting the spicule length of the male. This species also differs in the ratio of the muscular-glandular portions of the esophagus.

Rhabdochona cotti n. sp. (Figs. 1, 6)

Specific diagnosis: Rhabdochona. Slender worms, attenuated at both ends. Cuticle smooth, unstriated, of medium thickness. Anterior end rounded, pseudolabia rudimentary. Prostom concavoconoid, containing 14 minute teeth projecting anteriorly from short longitudinal ridges; no teeth at base of prostom; mesostom long, narrow, of constant diameter. Esophagus comparatively short, divided into anterior muscular and posterior glandular portions; glandular part not much thicker than muscular part. Deirids lateral, opposite midportion of stoma. Eggs embryonated when laid, 0.032–0.033 mm by 0.018–.020 mm with two long single (rarely double) filaments. Larval development in various mayfly and stonefly nymphs.

Female. Much larger than male and more opaque. Length 18.3–34.2 mm. Maximum width 0.19–.31 mm. Length of prostom 0.025–.036 mm; of total stoma 0.102–.143 mm; of muscular esophagus 0.374–.626 mm; of glandular esophagus 1.46–1.92 mm; of vagina 0.20–.46 mm; of tail 0.204–.350 mm. Width at base of prostom 0.042–.102 mm; at nerve ring 0.094–.148 mm; at end of esophagus 0.143–.202 mm; at vulva 0.190–.296 mm; at anus 0.070–.080 mm. Distance from anterior end to nerve ring 0.21–.43 mm; to excretory pore 0.305–.580 mm; to vulva 11.4–20.9 mm. Distance to vulva 55–65% of total body length.

Male. Length 7.65–10.6 mm. Maximum width 0.080–.131 mm. Length of prostom 0.017–.020 mm; of total stoma 0.082–.122 mm; of muscular esophagus 0.272–.340 mm; of glandular esophagus 0.80–1.28 mm; of tail 0.27–.36 mm. Width at base of prostom 0.030–.040 mm; at nerve ring 0.065–.078 mm; at end of esophagus 0.085–.110 mm; at anus 0.07–.11 mm. Distance from anterior end to nerve ring 0.17–.22 mm; to excretory pore 0.22–.32 mm. Right spicule 0.085–.120 mm long scoop-shaped, with rounded posterior end. Left spicule 0.309–.370 mm long, of which 0.163–.197 is the alate portion. Six pairs of postanal papillae, of which the second pair is sublateral and the others are subventral. About eight pairs of preanal papillae, of which the third pair anterior to the anus is sublateral and the rest subventral; of the four most anterior subventral pairs, the right member is usually more anterior than the left one.

Type host: Cottus cognatus.
Type locality: Griffith Springs State Fish Hatchery, Spokane, Washington.
Type specimens: U. S. N. M. Helm. Coll. No. 36991.

This species is described from one adult male and one adult female type specimens and seven male and four female paratypes. They were selected from more than one hundred adults preserved during the study. This species has been found in nearly one hundred per cent of the cottoids taken at the type locality, and occurs in about half of the cottoids (*Cottus cognatus, C. rhotheus, C. gulosus*) of the Little Spokane River and its tributaries. Infections range from a single worm to about 30 specimens in a single host. Twelve specimens of the same species of fish taken from Latah Creek, a distance of some thirty miles by water channels, were not infected.

R. cotti may be separated from other members of the genus by the eggs, which have long polar filaments, unlike those of *R. ovifilamenta* Weller, 1938, which attach to all parts of the surface and are comparatively very short. The females of *R. cotti* attain a size that is not duplicated by many others of this genus; of the forms which approach and equal its length, the glandular esophagus is much longer than in *R. cotti*. A comparison of the males shows that the spicule measurement and the pattern of preanal papillae is likewise distinctive.

The head of *R. cotti* is rounded much more than any other species observed; at the same time the pseudolabia are so reduced that they are almost indistinguishable. The body diameter is uniform for most of the length of the body, and the tapering toward the ends is much less prominent than in other species.

Rhabdochona pellucida n. sp. (Figs. 4, 5)

Specific diagnosis: Rhabdochona. Small, slender, transparent worms, attenuated at both ends. Cuticle smooth, thin, unstriated. Anterior end somewhat truncate; pseudolabia inconspicuous. Stoma heavily sclerotized; prostom cyathiform, containing 14 minute teeth projecting anteriorly from longitudinal ridges; four minute teeth at base of prostom; mesostom long, narrow, of constant diameter. Esophagus long, distinctly divided into slender, anterior muscular portion and thicker posterior glandular portion. Deirids lateral, opposite midportion of stoma. Eggs ovoid, embryonated when laid, 0.037–0.041 by 0.020–.024 mm; not filamented but covered with a thin gelatinous layer.

Female. Length 9.6–11.2 mm. Maximum width 0.20–.22 mm. Length of prostom 0.023–.026 mm; of total stoma 0.125–.147 mm; of muscular esophagus 0.322–.380 mm; of glandular esophagus 2.62–3 24 mm; of vagina 0.24–.36 mm; of tail 0.23–.26 mm. Width at base of prostom 0.031–.037 mm; at nerve ring 0.060–.085 mm; at end of esophagus 0.150–.205 mm; at vulva 0.170–.210 mm; at anus 0.073–.090 mm. Distance from anterior end to nerve ring 0.20–.30 mm; to excretory pore 0.306–.365 mm; to vulva 4.8–6.4 mm. Distance to vulva 50% to 59% of total body length.

Male. Length 3.98–5.3 mm. Maximum width 0.090–.120 mm. Length of prostom 0.017–.021 mm; of total stoma 0.102–.113 mm; of muscular esophagus 0.221–.295 mm; of glandular esophagus 1.43–1.87 mm; of tail 0.19–.32 mm. Width at base of prostom 0.018–.022 mm; at nerve ring 0.037–.051 mm; at the end of esophagus 0.085–.125 mm; at anus 0.060–.075 mm. Distance to nerve ring 0.135–.165 mm; to excretory pore 0.204–.244 mm.

Right spicule scoop-shaped, 0.092–.102 mm long, with reflected barb near tip. Left spicule 0.374–.482 mm long, of which 0.170–.190 mm is alate. About 11 or 12 pairs of preanal papillae, all of which are subventral except one sublateral pair opposite the third subventral pair from the anus. Six pairs of postanal papillae, all of which are subventral except the second pair from the anus, which is sublateral.

Type host: Pteichocheilus oregonensis.

Type locality Davis Lake, Washington.

Type specimens: U.S.N.M. Helm. Coll. No. 36994.

This species is described from adult male and female types and three male and three female paratypes, all taken from *Pteichochcilus oregonensis* from Davis Lake, Washington. Of fifteen fish from this source, nine have been infected with *Rhab-*

dochona pellucida. This parasite has also been taken from the same species of fish from Priest Lake, Idaho, and from Latah Creek, Spokane Co., Washington, and seems to maintain about a 60% infection in each of these areas. Altogether, about a hundred representatives of this species have been observed, taken from about 40 fish.

This is a rather small species, differing from many others in the total lengths of both males and females. All of the species within the size range of *R. pellucida* can be distinguished from it by the length and the shaft-blade ratio of the left spicule; most of these also differ in the preanal papillae. *R. oncorhynchi,* for which no male was described, differs considerably in the length of the glandular esophagus.

It seems to be most nearly related to *R. cascadilla,* with which it has much in common. They are both parasitic in cyprinids, and are very similar in general size and appearance. However, they can be distinguished by a careful comparison of the spicules which differ both in total length and in the blade-shaft ratio. Also, *R. pellucida* exceeds *R. cascadilla* not only in total length, but also in the measurements of the stoma, and muscular and glandular esophagus. It seems probable that further study will also show distinctions by comparing the intermediate hosts.

REDESCRIPTION OF *RHABDOCHONA CASCADILLA* WIGDOR, 1918

Rhabdochona cascadilla Wigdor, 1918 (Figs. 3, 7)

Specific diagnosis: Rhabdochona. Small, slender, transparent worms, attenuated at both ends. Cuticle thin, smooth, unstriated. Anterior end somewhat truncate; pseudolabia rudimentary. Prostom cyathiform, containing 14 minute teeth projecting from short longitudinal ridges; four minute teeth at the base of the prostom; mesostom long, narrow, of constant diameter. Esophagus moderately long, distinctly divided into slender anterior muscular part and thicker posterior glandular part. Deirids lateral, opposite midportion of stoma. Eggs embryonated when laid, 0.031–.038 by 0.019–.024 mm; not filamented but covered with thin gelatinous layer. Larval development in *Hexagenia* sp.

Female. Length 3.9–7.5 mm. Maximum width 0.116–.175 mm. Length of prostom 0.015–.026 mm; of total stoma 0.068–.116 mm; of muscular esophagus 0.177–.250 mm; of glandular esophagus 0.102–.197 mm; of vagina 0.15–.20 mm; of tail 0.16–.218 mm. Width at base of prostom 0.019–.022 mm; at nerve ring 0.046–.068 mm; at end of esophagus 0.102–.163 mm; at vulva 0.102–.140 mm; at anus 0.055–.065 mm. Distance from anterior end to nerve ring 0.102–.163 mm; to excretory pore 0.163–.210 mm; to vulva 2.19–4.09 mm. Distance to vulva 51% to 57% of total body length.

Male. Length 2.18–5.76 mm. Maximum width 0.085–.102 mm. Length of prostom 0.014–.019 mm; of total stoma 0.060–.085 mm; of muscular esophagus 0.11–.18 mm; of glandular esophagus 0.62–1.09 mm; of tail 0.17–.25 mm. Width at base of prostom 0.016–.019 mm; at nerve ring 0.034–.054 mm; at end of esophagus 0.078–.102 mm; at anus 0.068–.078 mm. Distance from anterior end to nerve ring 0.10–.12 mm; to excretory pore 0.15–.18 mm. Right spicule 0.065–.95 mm long, scoop-shaped, with a reflected barb on the left side near the posterior end. Left spicule 0.333–.383 mm long, of which 0.13–.16 mm is alate.

About 11 pairs of preanal papillae; all are subventral except the third pair from the anus which is sublateral; all pairs anterior to the third subventral pair from the anus have the right member more anterior than the left one. Six pairs of post-anal papillae, of which all are subventral except the second pair which is sublateral.

Author's specimens: U.S.N.M. Helm. Coll. No. 36993.

The specimens on which this description is based were taken from *Semotilus a. atromaculatus* and *Hyborhynchus notatus* from the Embarass River eight miles south of Urbana, Illinois. They were later compared with specimens identified as *R. cascadilla* by Dr. R. V. Bangham, who took them from *Notropus whipplii* from Lake Erie and from *Notropus cornutus, Catostomus commersonii, Semotilus a. atromaculatus,* and *Margariscus margarita nachtriebi,* all from Algonquin Park lakes

(Bangham, 1940). The original specimens taken by Wigdor from *S. atromaculatus* and *N. cayuga* from Cayuga and tributaries could not be located for comparison.

It was decided to consider this group conspecific with *R. cascadilla* Wigdor, 1918, because of the similarity of most characteristics. The size range and host relationship overlap, and many of the specific measurements such as the length of the longer spicule and the vagina also coincide. However, in some respects, the previous description differs radically from the present observations; comparison of the description and illustrations show the original publication was obviously in error. The drawing of the esophagus shows that the anterior part of the "esophagus" corresponds to the stoma, and the "second part" of the esophagus is really the muscular portion. Thus, the "distinctly short" esophagus mentioned by Wigdor would not be a valid characteristic. By comparison of the drawing of the longer spicule with the reference line, it can be seen that the spicule would not be 0.04 mm long but rather 0.4 mm. It is difficult to determine from the illustration whether the discrepancy in papillary pattern is actual, or may be a reflection of poor specimens or hasty observation.

DISCUSSION

It would appear from study of American members of this genus that the measurement and conformation of the longer (left) spicule is one of the characteristics most easily determined and at the same time most specific. The ratio of the shaft (or cylindrical) portion to the blade (or alate) portion should be considered; in the five species observed, this was a very constant value for each species. In all specimens examined, the left spicule was the longer one. There was no suggestion of a gubernaculum although the shorter spicule performs the same function. Fujita (1927) described two long spicules of equal length and an "accessory spine" for *R. salvelini*. This should be reinvestigated, with examination from a ventral view.

The presence of 6 pairs of post-anal papillae of which the second pair from the anus is sublateral, is a constant male characteristic in all the species studied by the author, and has been reported in many previous descriptions. This might well be a true generic characteristic. The asymmetry of the anterior members of the preanal papillae has not been mentioned before. This is difficult to determine in fixed specimens because of the usual caudal flexion, but may be seen by manipulation of live specimens. The right member of the pair is anterior to the left one where such asymmetry occurs.

Identification of a species from the female alone presents a very difficult problem, since they present very few specific characteristics. Filamented ova, as in *R. ovifilamenta* and *R. cotti*, are distinctive. Vaginal length appears to reflect the length of the long spicule, but varies considerably within each species. Dependence on host specificity, geographical range, and measurements of size are of less value as the number of described species increases, and their distribution away from the type locality is discovered.

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EXPLANATION OF PLATE.

FIGS. 1–4. Posterior ventral view of males. Same magnification: reference line 0.1 mm.

FIG. 1. *R. cotti*
FIG. 2. *R. decatureusis*
FIG. 3. *R. cascadilla*
FIG. 4. *R. pellucida*

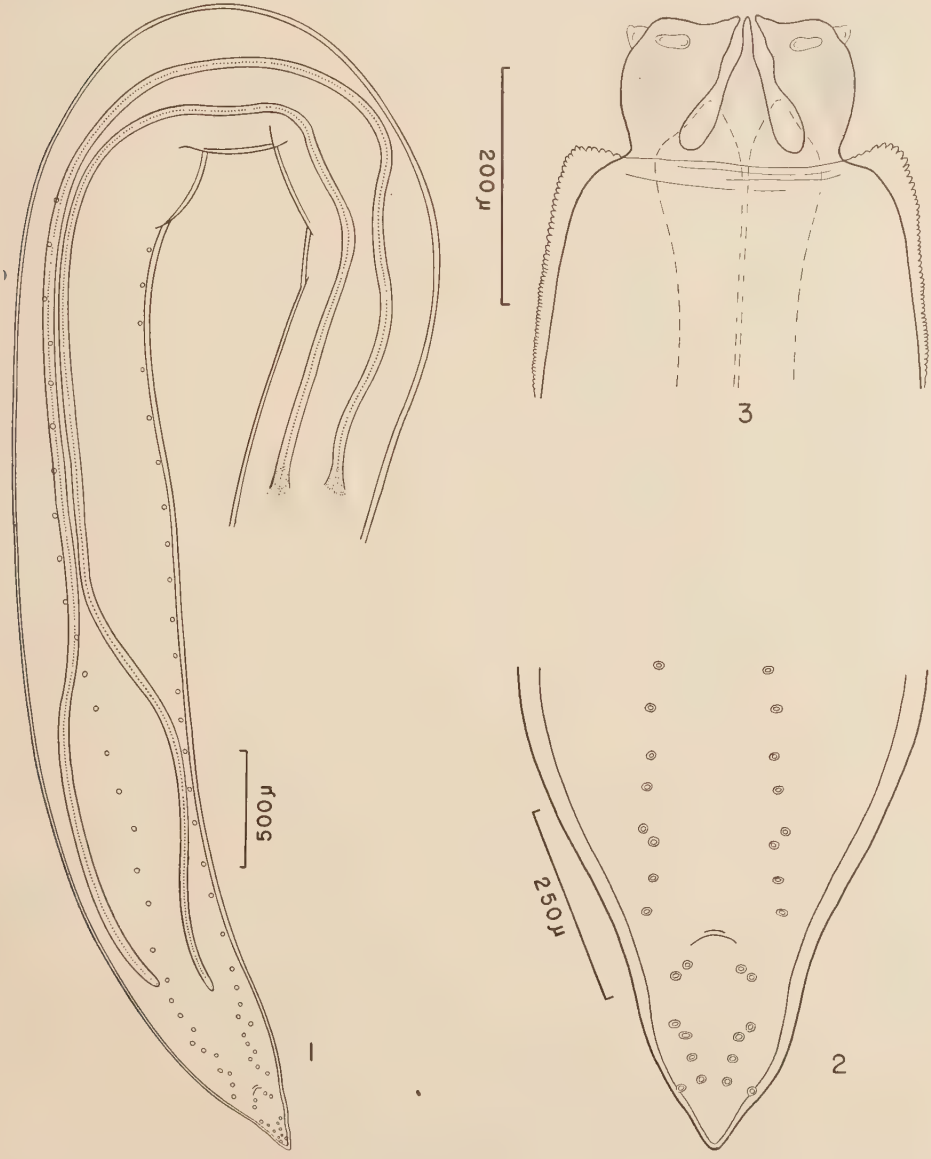
FIGS. 5–9. Spicules; same magnification: reference line 0.1 mm.

FIG. 5. *R. pellucida*
FIG. 6. *R. cotti*
FIG. 7. *R. cascadilla*
FIG. 8. *R. kidderi*
FIG. 9. *R. decatureusis*

RESEARCH NOTES

THE OCCURRENCE OF *CONTRACAECUM TRAVASSOSI* (NEMATODA) IN NORTH AMERICA

Seven specimens of a nematode (5 males and 2 females) of the genus *Contracaecum* were collected from the ventriculus of *Pandion haliaetus carolinensis* (Gmelin) (osprey) captured at Greenville, Ohio. After a comparison with *C. travassosi* described by Gutierrez (1943. Sobre la morfologia de una nueva especie de *"Contracaecum"* (Nematoda: Ascaroidea). Rev. Brasil.



FIGS. 1 to 3. *Contracaecum travassosi* Gutierrez, 1943. 1, posterior end of male showing extreme length of spicules; 2, ventral papillae arrangement of male tail; 3, latero-dorsal lips and interlabium.

Biol. 3: 159–172.) from the cormorant (*Phalacrocorax albiventer*) collected at the San José light-house, Territory of Chubut, Argentina, our material has been assigned to that species. So far as the writers have been able to determine, this is the first record of the species from North America and since no description of the species is available in North American literature a brief description is presented.

Males; body length 34 to 58 mm (average 42 mm); width 576 to 847 microns (average 739 microns), esophagus length 3.5 to 7.0 mm (average 4.6 mm), head-nerve ring distance 487 to 705 microns (average 615 microns); lip width 153 to 192 microns (average 163 microns); anterior end width 256 to 320 microns (average 281 microns); intestinal cecum length 2.1 to 4.4 mm (average 3.1 mm); cloaca-tail distance 256 to 384 microns (average 303 microns), left spicule length 7.5 to 12.9 mm (Fig. 1) (average 9.4 mm); right spicule length 7.2 to 11.4 mm (average 8.9 mm); 30 pairs of pre-anal papillae; 6 pairs of post-anal papillae, the pair nearest the cloaca are double (Fig. 2).

Females; body length 53 to 55 mm, width 1.1 to 1.3 mm; esophagus length 5.0 to 5.1 mm; head-nerve ring distance 750 to 760 microns, lip width 210 to 217 microns, anterior end width 375 to 384 microns, intestinal cecum length 4.0 to 4.3 mm; cloaca-tail distance 430 to 448 microns; anterior end-vulva distance 24 mm, eggs 62×43 microns. Two uteri. The anterior extremity (Fig. 3) has 3 labia and 3 interlabia arranged alternately.—Banner Bill Morgan, Everett Schiller and Robert Rausch, *Department of Veterinary Science, University of Wisconsin, Madison.*

NOTES ON THE COURSE OF A PINWORM INFECTION

A diagnosis of *Enterobius vermicularis* was made on a 43 year old male, 15 January 1947, and subsequently on the other four members of his family. None of the individuals experienced discomfort so the course of the infection was followed in the man.

The following procedure was established for this study. A daily Scotch tape swab was taken each morning before bathing or defecating. A piece of tape about 6 cm in length was applied to the perianal skin, once on each side, then anterior and posterior to the anus. Mineral oil was used in mounting and in all positive swabs counts were made on ten excursions across the tape under the low power of a microscope. The average number of eggs per excursion gave some idea of the intensity and was a valuable aid in determining periods of migration. In the earlier phase of the observations an attempt was made not to change daily habits, except that baths were usually taken in the morning after the swab had been prepared. On 27 May 1947 the patient left the U. S. for Japan. The rest of the family received treatment with gentian violet and lost their infections. Daily swabs were continued to see what effect removal from the family and other changes in environment would have on the infection. A change was made in the bathing routine soon after arrival in Japan because the weather was very hot. Baths were taken at least once a day and sometimes as many as three were taken in a 24 hr. period. Swabs were examined until 31 March 1948. During this period of 503 days, examinations were not made on nine days. These were scattered throughout the period of study and could not change the results materially.

The observations show three phases in the infection: (1) the period while in the family environment; (2) a transitory period shortly after the above; (3) a period when the infection was barely maintained, until it finally disappeared. During the first period, 15 January to 31 May 1947, worms descended and laid eggs 63 out of 137 days. The average number of eggs per excursion across the tape was 59.7. In June and July, the transitory period, worms descended only 17 out of 61 days, with an average egg count of 54.2. During the first period there was an average of 13.6 days per month that worms descended. In the transitory period it was 8.5 days. The last time eggs were laid occurred on 31 March 1948. During the period of August through March worms descended only 12 times, or an average of 1.5 times per month. The average egg count was 44.4.

During the first and second periods there was no discernible rhythm in the time the worms appeared. In the third phase the reduction of the infection resulted in a noticeable periodicity. The intervals between migrations, from the end of one to the beginning of another, varied from 25 to 43 days.

The only symptoms noticeable during the entire course of the infection were some itching and occasionally the crawling of a worm could be felt. The presence or absence of symptoms was recorded before the slide for the day was examined. During the period of infection symptoms and eggs were recorded 53 times. No symptoms, but the presence of eggs, was reported 38 times. Symptoms, but no eggs, were recorded 12 times.

In this case there was no evidence of hyperinfection which agrees with Swellengrebel and Schüffner (1946, Nederl. Tijdschr. v. Geneesk., 90: 762–764). Although descent of worms often

occurred from two to eight consecutive days there was no evidence that the female worms returned to the colon, as suggested by Madsen (1945, Acta Path. et Microb. Scand., 22: 392–397).—Donald B. McMullen, *Section of Medical Zoology, 406th Medical General Laboratory; and the Department of Preventive Medicine and Public Health, The University of Oklahoma Medical School.*

HETERAKIS GALLINARUM (SCHRANK, 1788) *NEC HETERAKIS GALLINAE* (GMELIN, 1790)

During a detailed work on the systematics of even common poultry nematodes it turned out that many systematic and nomenclatural errors still are circulating. This was the case with the species of *Capillaria* [Holger Madsen (1915)] and also with the common *Heterakis* of fowl, etc.

Most authors in referring to this parasite follow the lead of Cram (1927, p. 52) in using the name *Heterakis gallinae* (Gmelin, 1790). This name is taken from a paper by Freeborn (1923), a brief report laying the main stress on control of the parasites. The current names until that time were *H. vesicularis* (Froelich, 1791) and *H. papillosa* (Bloch, 1782), the latter one due to a misdetermination by Railliet (1885).

Gmelin (1790, p. 3034) gives the name *Ascaris gallinae* with reference to Goeze (1782, p. 86), one of the "ascarides teretes" of this author. Already this reference contains an error, since Gmelin indicates the seat of the worm as the ceca although Goeze in the place cited does not mention this, but refers to a figure (pl. I, fig. 7) which undoubtedly, and generally accepted, is a species of *Ascaridia,* probably *A. galli* (Schrank, 1788) (compare Baylis (1932, 1936). Further he has the same reference (p. 86) under his *Ascaris galli,* and evidently in the descriptions has confused the indications of Goeze. In pl. I, fig. 4, Goeze illustrates a worm which undoubtedly is that dealt with here. The common appearance is that of a *Heterakis,* and not an *Ascaridia,* with a long, slender caudal tip and sharply-pointed spicules. The bend near the tip of one of them, without any doubt to anyone familiar with the species (a rare case in the old helminthological literature!) demonstrates that it is the common *Heterakis* of poultry, etc. It must be granted that in connection with the legend for this figure Goeze (p. 76) states that the natural size of the roundworm of the cock is as shown in his tab. I, fig. 6, which illustrates an *"Ascaris"* (*Ascaridia*) from a pigeon even being larger than the above mentioned fig. 7 (*Ascaridia*). His own indications of the magnification suggest, however, that the real size has been somewhat below 4 mm. It is therefore clear that the great size indicated on p. 76 is erroneous. The specimen of Goeze, being the common *Heterakis* of poultry, is mentioned by Gmelin under the name *Ascaris galli,* which name also in his paper comprises specimens of Goeze, which are, however, *Ascaridia galli* (Schrank, 1788). Referring to Goeze (1782, p. 76, pl. I, fig. 4), Schrank (1788, p. 9) correctly associates description and figure of Goeze under the name *Ascaris gallinarum.* Therefore on the basis of priority, the proper name for the *Heterakis* of fowl, etc. should be *Heterakis gallinarum* (Schrank, 1788), and not *Heterakis gallinae* (Gmelin, 1790).—Holger Madsen, *Jagtraadets vildtbiologiske Undersøgelser. Nørregade* 10, *Copenhagen.*

MALARIA IN THE ENGLISH SPARROW

As a result of a survey of 516 birds, data were accumulated concerning the incidence of infection, and seasonal and geographic distribution of *P. elongatum, P. cathemerium* and *P. relictum* in the English sparrow. It is the purpose of this paper to summarize these data.

The majority of studies dealing with the blood parasites of birds have been confined to comparatively limited areas and have been carried out with many different avian hosts most of which are migratory. Likewise, the birds obtained for many of these investigations were all taken during one season of the year, generally in the summer. The present study is concerned with a single, non-migratory species, the English sparrow. For the most part this species has constituted a very minor part of most surveys; however, a few studies have included a considerable number of them. Manwell and Herman (1935, Amer. J. Trop. Med., 15: 661–673) found six of 244 English sparrows infected with *P. relictum.* Huff, (1939, J. Amer. Vet. Med. Assn., 94: 615–620) in a survey of several states found thirteen of 125 infected with plasmodia although he cautioned against interpreting the findings as natural incidences since the sampling was not random. Jordan (1943, J. Parasit., 29: 260–263) found malarial parasites in 70 of 489 English sparrows trapped in Georgia.

Material for the present study consisted of blood films which had been collected over a period of several years from twenty-five states. The writer is indebted to Dr. John B. Calhoun for the loan of those taken from birds from 1939 through 1944. In all, smears from 516 English sparrows were examined. These were stained with Giemsa stain and each was examined approximately

twenty minutes except when duplicate smears were studied. One hundred and ninety-five of the birds were caught in traps in Baltimore during the summer of 1947 and these were kept in the laboratory for several weeks for further study and as a source of new strains.

An examination of blood smears taken from the 516 English sparrows revealed an infection with plasmodia of 11.2 per cent. The distribution of these forms is shown in Table 1. *P. relictum* was by far the most predominant parasite and was present in 31 of the smears. Five birds harbored *P. cathemerium* and four were infected with *P. elongatum*. Of the 18 infected with unidentified parasites, 14 carried forms which displaced the red cell nucleus while the remaining infections were of a type with elongate gametocytes. A single sparrow with both *P. relictum* and *P. elongatum* was the only bird haboring more than one species of parasite as judged by blood examination. The monthly incidence of infection is shown in Table 2. It was quite low with the

TABLE 1.—*Distribution of Plasmodia in the English Sparrow*

State	No. examined	P. relictum	P. cathemerium	P. elongatum	Unidentified
Arkansas	18	2	1		
Kansas	41	1			3
Maine	10		1		
Maryland	210	20	2	2	5
Michigan	19	1			
Minnesota	11	1			2
N. Dakota	15				3
N. Jersey	25	1			
N. Mexico	23			1	4
Ohio	17		1		
S. Carolina	5	2			
Texas	9	1			
Virginia	53	2		1	1
Total	456	31	5	4	18

TABLE 2.—*Monthly incidence of malaria in the English Sparrow*

Month	No. examined	No. positive	% positive
January	33	0	0
February	33	0	0
March	57	2	3.5
April	42	7	16.6
May	23	5	21.7
June	42	5	12.0
July	19	4	21.0
August	30	3	10.0
September	160	24	15.0
October	35	5	11.0
November	16	1	6.2
December	26	2	7.7
Total	516	58	11.2

beginning of winter and sparrows captured during the months of January and February were completely parasite-free. Following a very low incidence in March, the level of infection rose and in general remained high during the spring and summer months.

While a review of the literature reveals an average incidence of approximately 7 per cent malarial infection in a total of 9577 birds of many different species studied in twenty-four blood parasite surveys, 11 per cent of the birds in the present study, although non-migratory, were infected with malarial parasites. Furthermore, of the 195 sparrows captured in Baltimore 14 per cent harbored plasmodia. Manwell and Herman (1935, J. Parasit., 21: 415–416) have stated that migratory birds are much more commonly infected with blood protozoa than those which do not migrate and they found (1935, Amer. J. Trop. Med., 15: 661–673) comparatively few English sparrows and starlings, both non-migratory species, infected with malarial parasites. On the contrary, Jordan (1943) in a larger study, found that over 16 per cent of the English sparrows examined were infected with plasmodia. Her results are substantiated by those of the present study, particularly in view of the fact that birds included in her survey were trapped from May through October, during which time the level of infection is highest. It therefore seems that the incidence of malaria in at least one non-migratory species may not be as low as previously believed and that conclusive evidence in this regard awaits further studies on larger numbers of non-migratory species.—DON W. MICKS, *School of Hygiene and Public Health, Johns Hopkins University.*

THE OCCURRENCE OF BAT MALARIA IN THE NEW HEBRIDES AND PHILIPPINE ISLANDS

In a survey at Espiritu Santo, New Hebrides, and on Luzon, Philippine Islands, four out of five species of bats were found to be infected with species of *Plasmodium*. Ninety-two fruit bats of the species *Pteropus geddiei* and *P. eotinus* were examined in the New Hebrides. All were infected with parasites fitting the description of *P. pteropi* (Breinl, A. 1911, Aus. Inst. Trop. Med. Rep.) and represent new host records for this parasite. One hundred specimens of NYCTERIBIIDAE which infested all the bats studied were dissected but no malaria organisms were found. Parasites in the blood stream were similar to those described by Manwell from bats of New Guinea (1946, Am. J. Hyg. 43: 1–12), except that no segmenting forms or exoerythrocytic forms were found. Parts of spleen, liver, bone marrow, brain, and lung were fixed in ten per cent formalin, sectioned using the celloidon technic, and stained with Maximow's eosin-azure-haemotoxylin stain. No segmenting forms were found in any of these organs. It is interesting to note that in spite of effective mosquito control in the area studied, one hundred per cent of the bats examined throughout a year period were infected.

Six out of fifty specimens of *Miniopterus australis* were infected with a parasite identical with the species described by Dionisi as *P. murinum* (1899, Arch. Ital. di. Biol. 31: 151-152). This constitutes a new host record. Sexual forms only were found. One hundred bats of the species *Hipposideros cervinus* were examined with negative findings.

Only one unidentified specimen of PTEROPIDAE was examined at San Fernando, La Union, Philippine Islands. It was infected with *P. pteropi*.—R. BARCLAY MCGHEE. *Rockefeller Institute for Medical Research, Princeton, N. J.; formerly Captain in the Sn. C., A. U. S.*

CUTANEOUS MYIASIS DUE TO *CHRYSOSTOMOMYIA BERGI* (BLANCHARD)

FIRST REPORT FROM THE ISTHMUS OF PANAMA

The writer (1947, Arch. Hosp. Santo Tomás, 2: 11–17) studied the incidence and epidemiology of cutaneous myiasis on the Isthmus of Panama and the types of larvae found. He later (1948, J. Parasit. 34: 343–344) reported the first case of cutaneous myiasis on the Isthmus caused by *Stephanostoma haemorrhoidalis* (Fall) = *Sarchophaga haemorrhoidalis* and *Cochliomyia hominivorax* (Coq.). This paper reports a new case of cutaneous myiasis due to *Chrysostomomyia bergi* (Blanchard), a species which, furthermore, has never been reported as producing cutaneous myiasis in man.

Report of the case: A. L. M., a two-month old black female was referred to the writer at the Dispensary of the Santo Tomás Hospital by Dr. J. Feraud Peñfiel with a diagnosis of myiasis of the scalp. She was hospitalized (Record #338383) in January, 1946. On local examination a fetid, crateriform ulceration of 4×4 cm. was seen on the right parietal area. It was covered with larvae which, on removal, left an irregular surface. The periostium was not perforated. Seventy-two larvae were removed, forty of which were bred out for study and classification. Both larvae and adults were sent to Prof. M. E. Jörg, who classified them as *Chrysostomomyia bergi* (Blanchard). The material sent by us has also been studied by Prof. E. E. Blanchard, who verified the identification. The species was described by him (1939, Physis (Rev. Soc. Argent. Cien. Natur. 17: 791–856).—CARLOS CALERO M. *Medical Department, Panama Hospital, Panama, R. P.*

NOTES ON SOME FLEAS FROM RODENT NESTS IN NEW MEXICO

Since most investigators collect and study fleas from host animals only, little attention has been directed to the fleas found in underground nests of rodents. This possibly results from the impression that individuals of all the flea species parasitic on a given host may be taken eventually from the host. In addition, there are the difficulties encountered in excavating underground nests and removing the rodents from the nest materials. Hubbard (1947, Fleas of Western North America, The Iowa State College Press) advocates the removal of fleas from rodent nest materials by picking the nest apart and thus removing the fleas. As he indicates, this is a very tedious task. In our work with fleas in the Albuquerque, New Mexico, area, we have utilized Berlese funnels with excellent results. In this device, the nest material is spread on a screen in a metal funnel. Below the funnel at the apex is a container of alcohol into which the fleas drop when they move about in an effort to avoid the light and heat of an incandescent lamp suspended over the open end of the funnel.

So far, by use of our Berlese funnels, we have removed and studied the fleas from four nests of the hoary wood rat (*Neotoma micropus canescens* Allen) and from one nest of the New Mexico banner-tail kangaroo rat (*Dipodomys spectabilis baileyi* Goldman). All five nests were

taken from the "mesa" a few miles east of Albuquerque and near the base of the Sandia Mountains. A resume of the fleas taken in the wood rat nests is given in table 1. In the kangaroo

TABLE 1.—*Numbers of fleas of various species collected from four nests of the hoary wood rat* (Neotoma micropus canescens)

Species of flea	Nest collection number and date (1948)			
	1 Feb. 2	2 Oct. 10	3 Oct. 10	4 Oct. 10
Orchopeas sp.	0	1	2	0
Anomiopsyllus sp.	188	6	32	901
Megarthroglossus sp.	7	3	1	9
Total fleas from each nest	195	10	35	910

rat nest, taken on March 6, 1948, we found 117 fleas belonging to three species. Sixteen of the fleas were of an undescribed species and genus related to *Rectofrontia* and *Actenophthalmus*. In the nest were found five individuals, three males and two females, of an apparently undescribed species of *Thrassoides* and 96 fleas belonging, we believe, to a new species of the genus *Meringis*. An examination of the food storage from the same nest gave us 11 individuals, three males and eight females, of the new species of *Meringis* and one female of the undescribed species of *Thrassoides*. Our study of the fleas from the kangaroo rat nest as well as our study of the fleas from the wood rat nests indicates the possibilities of discovering hitherto unknown species of fleas in rodent nests.

Our collections clearly demonstrate that some of the fleas studied are confined almost exclusively to the nest and seldom or never leave the nest on the rodent host. Although we found great numbers of certain species of fleas in nests, the same fleas were found rarely or at least not in appreciable numbers on the host animals taken by either snap or live trapping in the same area. A confinement of fleas to the nest is especially conspicuous in our species of *Meringis* from the kangaroo rat nest and the species of *Anomiopsyllus* from the wood rat nests.

During our study, we have noticed a peculiar sex ratio in some of the species of fleas investigated, this being especially marked in the fleas that are individually abundant. The number of fleas segregated according to sex and the sex ratio of four species are indicated in table 2.

TABLE 2.—*Sex ratios of species of fleas collected in sufficient numbers to validate results*

Species of flea	Host	♂♂	♀♀	Total	Ratio $\frac{♀♀}{♂♂}$
Meringis sp.	Kangaroo rat	27	71	98	2.6+
New gen. and sp.	Kangaroo rat	8	8	16	1.0
Anomiopsyllus sp.	Wood rat	395	732	1127	1.9-
Megarthroglossus sp.	Wood rat	10	10	20	1.0

In the instance of the *Anomiopsyllus sp.* we found the females nearly twice as numerous as the males in all four nests investigated. We have no definite information relative to an explanation of this unequal sex ratio but we suggest that the females may have a longer life span than the males.—C. CLAYTON HOFF AND LELIA A. WILLIAMS, *Department of Biology, University of New Mexico, Albuquerque, New Mexico.*

A NEW HOST FOR *PLATYNOSOMUM FASTOSUM* KOSSACK, 1910 (TREMATODA, DICROCOELIIDAE)

Platynosomum fastosum is a common parasite trematode of the liver of the domestic cat (*Felis catus domesticus* L.) and it has large geographical distribution including Malaya, Brazil (S. Paulo, Rio and Pernambuco), Puerto Rico and North America. It has been obtained from the liver of *Oncoides minuta* (Temm.) and *Grison vittata* (Schreb).

The biology of *P. fastosum* was worked out by J. F. Maldonado (Puerto Rico J. Publ. Health & Trop. Med., 21; 17–39, 1945). The life cycle is accomplished through a land snail (*Subulina octona*) and a second intermediate host, a lizard (*Anolis cristatellus*).

In 1943, we found in the liver of a wild cat, *Herpailurus yaguarondi yaguarondi* (Lac.), killed in the interior of the State of Pernambuco (Brazil) and determined by P. Sawaya, a great number of *P. fastosum*. These specimens are much larger than the common parasites from the domestic cat of the same region, but they were morphologically indistinguishable.

Specimens of medium size measured 9.5 mm long and 3.0 mm wide; these values are higher than those given by Travassos (Revisão dos Dicrocoeliidae, 1944) who agreed with us in the determination of the species.

The authors are indebted to Dr. P. Sawaya and Dr. L. Travassos for helpful suggestions.—F. A. Simoes Barbosa and C. Pontual, *Medical School (Dept. Parasitology), University of Recife, Brazil.*

VAGINAL SPHINCTER, ORGAN OF *ECHINOCOCCUS GRANULOSUS* (BATSCH, 1786).

In studying the anatomy of the genital apparatus of cestodes that parasitize man in either adult or larval stages, we have demonstrated the presence of a muscular sphincter at the level of the vagina in *Echinococcus granulosus.* It has been possible to distinguish this organ by means of new staining techniques utilizing azocarmin B and silver impregnations. Although azocarmin B is a well known stain, it has not been used to stain "parasites in toto". This stain clearly shows the organ described. At approximately 100 microns from the edge of the proglottid a deeply stained muscle, about 15 microns in width, can be seen encircling the vagina. This muscle when contracted, obliterates the lumen of the vagina; distally, the vagina continues, sometimes with dilatations, and then narrows to empty in the seminal reservoir.

Having found this organ in specimens of *Echinococcus granulosus* stained "in toto," we tried to identify its histological structure in sections of the parasite. Serial sections showed a muscular band encircling the vagina and forming a sphincter.

It is recognizable in the second and third proglottids, specially in the latter, and in both spontaneous and experimental infections of the dog.

It appears that this organ prevents the egress of the penis from the vagina at the moment of fecundation and the escape of the spermatozoa that could be lost in the external part of the vagina.—J. Bacigalupo and E. Rivero, *Institute of Parasitology, School of Medicine, Buenos Aires, Argentina*

INTERNAL PARASITES OF CENTRAL NEW YORK MUSKRATS

(*Ondatra z. zibethica* L.)

During the winters of 1945–46 and 1946–47, muskrat carcasses were collected from trappers of Hamilton, Madison County, New York. While they were collected primarily for skeletal material, many were also examined for internal parasites. The information obtained adds to the scanty distributional records at present available for the species found.

The class Trematoda is represented by six species, three of which, *Notocotylus quinqueserialis, Echinostoma revolutum,* and *Plagiorchis proximus,* were found in numbers in 25% or more of the muskrats examined.

Notocotylus urbanensis (Cort): Of 40 muskrats examined in January and February 1947, only three (7.5%) had infections; in each case the worms numbered over 25. They were concentrated in the proximal portion of the large intestine and were closely applied to the wall. The intestinal ceca were invariably bright red (filled with blood).

Notocotylus quinqueserialis (Barker and Laughlin): This fluke was the most frequent and abundant parasite. While no record was kept for this form during the first winter, during the second winter, 47 out of 53 (89%) 'rats' had this fluke in numbers. They were found without exception in the caecum, often with the intestinal ceca bright red with blood. More than 10 individuals of this fluke were found in each of 77% of the muskrats examined.

Meadow mice (*Microtus pennsylvanicus* (Ord)) caught in the same area were all infected with this species. The forms from meadow mice are very different in general appearance, giving the impression of being larger and thicker.

Pseudodiscus zibethicus (Barker and East): Three specimens of this large fluke were found during the winter of 1946–47, usually in that region of the caecum into which the small intestine empties. Two were found in one muskrat, one in another.

Echinostoma revolutum (Froelich): This large echinostome, a close second in frequence and abundance to *N. quinqueserialis,* occurred in 89% of the muskrats examined during the first winter, and in 76% examined during the second winter. It was found throughout the small intestine, the larger (adult) specimens generally in the jejunum and ileum, while the immature forms were concentrated in the duodenum, near the pyloric sphincter. *Echinostoma revolutum* occurred in numbers greater than ten in each of 36% of the muskrats examined.

Opisthorchis tonkae Wallace and Penner: 20 specimens of this opisthorchid were found in the duodenum of a muskrat collected in 1946. This is not the normal habitat of the fluke. The

carcass may have been handled roughly enough during skinning to dislodge them from the bile duct. However, the bile duct, gall bladder, and pancreas were all carefully examined in all muskrats and no other specimens of the fluke were found.

Plagiorchis proximus Barker: 14 of 53 (26%) muskrats contained this small fluke. It was usually found in the duodenum, occasionally in the lower portions of the small intestine. Generally *P. proximus* was found closely appressed to the intestinal wall, often being difficult to remove. In 12% of the muskrats, each containing 10 or more specimens. This form was favorable for live study under the microscope.

All muskrats were examined in vain for schistosomes.

The class CESTODA was represented by four forms, of which only two could be identified with a reasonable degree of accuracy.

Hymenolepis evaginata Barker and Andrews: This tapeworm was found in 6 of 53 (11%) muskrats in the following numbers—1, 1, 3, 3, 4, and 5 specimens. It was also found in *Microtus pennsylvanicus* from the same area.

Taenia taeniaeformis (Batsch): The livers of 3 of 53 (6%) muskrats each had a single cysticercoid of this tapeworm (*Cysticercus fasciolaris*).

22% of the muskrats, and a greater percentage of the meadow mice, had several to numerous cysticeroids of an unidentified tapeworm. The cysts were small, measuring 3 to 5 mm. in diameter, and were located at or near the periphery of the lobes of the liver. Also noted, and likewise unidentified, were three large sausage-shaped coenuri on the mesenteries of the small intestine. The largest measured 15 by 30 mm. One muskrat had a large quantity of fragments of a large tapeworm(s) in its caecum.

The phylum NEMATODA was represented by only one form, the muskrat trichurid, *Trichurus opacus* Barker and Noyes. It was found only twice. It is easy to miss, inhabiting the caecum which generally is filled with black mud-like chyme, and it may well be commoner than it seemed.

The adults of the dog heart worm (*Dirofilaria*) were looked for but none were found.

It was interesting to note that *Notocotylus quinqueserialis* and *Hymenolepis evaginata* occurred in both the meadow mouse and in the muskrat. The small cysticercoids mentioned above likewise occurred frequently in both hosts, and even the cysticercus of *Taenia taeniaeformis* was found once in the meadow mouse. However, the common echinostome of the local muskrat was never found in the meadow mice.

I wish to thank Dr. Charles E. Foster, Professor of Biology at Colgate University for his help in this project. Mr. Howard Bradley of Hamilton, New York, very kindly contributed the greater part of the material examined. All of the determinations were made by the writer. Some of the material is available to workers upon request.—ROBERT L. EDWARDS, *Biological Laboratories, Harvard University, Cambridge 38, Mass.*

ERRATA

JOURNAL OF PARASITOLOGY, Volume 35, Number 3.

Page 273, for line 5, substitute:

..... nal line may give rise, ultimately, to many thousands of cercariae. The number of .....

Page 323, for line 3, substitute:

During the tenure of his first professional appointment as Protozoologist at the .....

Page 323, for line 8, substitute:

..... in Munich and undertook a number of scientific expeditions to Africa and the Middle .....

AMERICAN SOCIETY OF PARASITOLOGISTS

Preliminary Announcement of the Twenty-Fourth Annual Meeting

Tuesday, Wednesday and Thursday, December 27–29, 1949
New York, New York

Following recommendation of the Council and action taken by the Society at the 1948 meeting in New Orleans, the American Society of Parasitologists will meet with the American Association for the Advancement of Science in New York City in 1949. The Hotel Statler will be the official headquarters for the Society and functions will be divided between the Hotel Statler and the Columbia University campus, 116th Street at Broadway.

PROGRAM

December 27 (Tuesday)	—morning	Regular session
	afternoon	Regular session
	evening	Dinner and Council meeting
December 28 (Wednesday)	—morning	Symposium
	noon	Luncheon
	afternoon	Demonstration and tea
December 29 (Thursday)	—morning	Regular session
	afternoon	Regular session

All members are urged to attend this convention since it will present an unusual opportunity to meet members of the American Society of Parasitologists, members of the American Society of Zoologists, and members of other societies affiliated with the A.A.A.S.

Respectfully,
H. W. Brown, *Secretary*